TRACHEAL WASH, BRONCHOALVEOLAR LAVAGE & TRANSTRACHEAL ASPIRATE

Collection Techniques

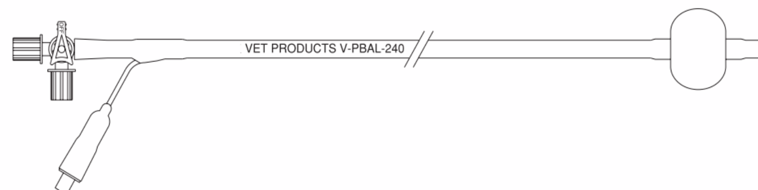
1. Tracheal Wash

A TW is usually collected via a catheter in the biopsy channel of an endoscope. Various specialised catheters are available to reduce the risks of contamination of the sample although the following method is simple and effective using a non-specialised plastic catheter.

- Restrain the horse. In many cases a nose twitch is perfectly acceptable for the TW procedure and avoids the costs and drug withdrawal issues associated with sedation. However, there are significant advantages of sedation if the operator/handler are inexperienced, the horse is difficult or if subsequent BAL is envisaged.
- Prior exercise (ridden or lunge) is preferred by many to encourage movement of lower airway secretions into the trachea. If subsequent BAL is planned then there is probably no point in doing this.
- Insert a sterile plastic catheter into the biopsy channel of a clean endoscope and advance the catheter until it is within an inch or two short of the end of the endoscope.
- Pass the endoscope (at least 1.1m and preferable 1.4m long) via the ventral or middle nasal meati and into the pharynx. Make note of any pharyngeal abnormalities (e.g. epiglottis, GP ostia, lymphoid hyperplasia). In many horses with airway disease, the larynx is very sensitive to endoscopy and persistent coughing during the TW procedure will result in loss of the sample. Therefore in many cases (especially with a strong prior suspicion of significant airway disease) it is worth desensitising the larynx with 5 ml local anaesthetic solution injected via the catheter before proceeding after waiting 1-2 minutes. Ideally this should be performed while the head is held as low as possible so that the local anaesthetic runs back out through the nose rather than down into the airways (carrying pharyngeal bacteria with it).
- Pass the endoscope through the larynx into the upper trachea. With the endoscope a few inches into the upper trachea make note of the quantity and nature of tracheal mucus deposits. If the horse is sedated then ensure the head is held reasonable elevated before proceeding or the sample will be lost. The catheter is then advanced out of the biopsy channel and 20-25 ml warm sterile saline is squirted onto the tracheal mucosa. This will be seen to flow distally and collect in a pool at the level of the thoracic inlet.
- The endoscope is advanced to within a few inches of the pool of fluid (not dipping into it as the endoscope will be contaminated with pharyngeal bacteria) and it is aspirated via the catheter. Virtually all fluid should be collectable but it doesn't matter if some is left behind. In normal horses the collected fluid should be clear with no more than a few small flecks of grey mucus.
- After collection of the TW sample then the endoscope is passed further to the carina making further note of distal tracheal/bronchial secretions. Other points of note are how sensitive/insensitive the carina/proximal bronchi are to the endoscope (this appears to correlate with degree of airway disease) and also how rounded/sharp the septum separating the left and right bronchi is (perhaps reflecting airway inflammation although a recent study casts doubt on this). If a BAL is also to be collected then a further 20ml local anaesthetic is sprayed into the right bronchus (and then collected from the trachea on withdrawal)

2. Bronchoalveolar Lavage

The technique can be performed with an uncuffed tube or via a 2 metre endoscope although failure to seal the airway with an inflatable cuff likely leads to more proximal airway sampling and therefore a greater probability of 'bronchial lavage' rather than bronchoalveolar lavage. Bronchial samples are intermediate in cytological variability between BAL (least variable) and TW (most variable) and are therefore less useful than BAL. This author prefers a specifically designed cuffed BAL tube (2.4 or 3.0 m long and 10mm diameter – e.g. Cooks) without endoscopic guidance (following prior endoscopy and TW as described above) that can be sterilised and reused. With a "blind" and unguided technique then it is to be expected that the caudodorsal right lung will be almost invariably sampled. If more targeted sampling is required (e.g. suspected left lung or ventral pathology or perhaps a repeat BAL procedure when the original results were unexpectedly normal) then this author would certainly favour an endoscopically chosen area of lavage performed via the endoscope biopsy channel. Lavage from the left lung may have more mast cells than the right.



Proprietary 2.4m long cuffed silicon BAL tube

If thoracic radiographs are intended to be taken then this should be done prior to the BAL procedure or a caudodorsal interstitial/alveolar infiltrate will be seen where the procedure was performed. Prior exercise makes no difference to BAL results.

Coughing (sometimes quite violent) is to be expected during the procedure (warn the owner if present). Apart from being unpleasant for the horse (and owner) this may lead to loss of the cuffed seal and collection of a bronchial lavage as described above. It is important to take procedures to limit the coughing if the samples are to be collected successfully (see below) but it is rare for the technique to be performed with no associated coughing. Horses with moderate to marked clinical signs of airway disease should probably not be subject to BAL until their clinical signs are mild to moderate.

- All horses should be well sedated (e.g. 0.3-0.4 ml detomidine plus 0.5-1.0 ml butorphanol) prior to the procedure. Bronchodilation with iv clenbuterol or atropine or inhaled salbutamol or iptratropium might also facilitate the technique and reduce coughing.
- Desensitise the larynx and carina with topically applied local anaesthetic (as described above under TW). If an endoscope is unavailable then the local anaesthetic can be applied via the BAL tube itself when the tube reaches the carina. This point is usually obvious as it characteristically triggers off a marked coughing response. After applying the local anaesthetic wait a few minutes before continuing with the procedure.
- Prefill 6 x 50 ml syringes with warm sterile saline
- Insert the BAL tube (or endoscope) naso-tracheally until significant resistance to further passage is encountered and it is wedged into a small (usually 4th or 5th generation) bronchus. Inflate the cuff with 5ml of air. Most horses will be coughing at this point and it is worth waiting for 20-30 seconds for coughing to subside.

- Sequentially attach the syringes and squirt the warm saline through the BAL tube into the distal airway. A pony may require only 150-250 ml but most horses require 250-500 ml. End-expiratory lung volume in most horses is approximately 60 ml/kg bodyweight (e.g. 30,000 ml for a TB-sized horse illustrating the inconsequential volume injected). Consistency of the volume used is important for interpretation of results. Some describe injecting and aspirating after each 50 ml syringe although this author injects the entire volume before reaspiration. Lower volume samples may be found to have more mast cells than high volume samples and more mast cells are usually seen in the first aliquot collected compared to later ones. It is therefore important to pool all collected fluid rather than just randomly selecting one sample for laboratory analysis.
- Immediately following the last syringe of saline, the same syringes are then used to 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. In a normal horse the fluid should be clear with low turbidity and no more than a trace of flocculent mucus within it. If there is an absence of stable foam then the technique should be repeated.
- It is customary to resume light exercise 24-48 hours after the procedure and more strenuous exercise may commence from 48-72 hours.

TW or BAL samples should be placed into EDTA for cytology and also plain tubes for culture. Cytology appears amenable to analysis and interpretation even after delays of 48-72 hours so fixation is usually unnecessary. However, given that relatively large volumes of fluid are collected by both techniques then there is normally plenty to permit dispatch of both fresh samples and samples fixed by adding a similar volume of alcohol or just a few drops of formalin. Samples can simply be centrifuged and smears made from the cell pellet or preferably cytocentrifuged which generally results in better preservation and easier identification of cells (become flatter). Standard and cytocentrifugation techniques may differ slightly in terms of more eosinophils, mast cells and macrophages using the latter technique.

3. Transtracheal aspiration

Transtracheal aspiration (TTA) is a less commonly used means of achieving a bacteriologically uncontaminated sample of tracheal secretions and is, theoretically at least, the most appropriate method of investigating horses with infectious respiratory disease.

- Restrain the subject with sedation or twitch.
- Clip and scrub an area centred one third of the way down the trachea from the larynx where tracheal rings can be felt.
- Inject 2ml local anaesthetic subcutaneously and make a small stab incision with a no 11 or no 15 blade. Pre-measure the distance from the site to the thoracic inlet (eg. 60cm).
- A 10 g TTA trocar is inserted between tracheal rings and the needle removed to leave the "sleeve". The TTA catheter (e.g. 70cm, 12-16 gauge) is passed through the "sleeve" into the airway to the level of the thoracic inlet.
- Inject 30 ml sterile saline and immediately reaspirate (move catheter in/out if none is obtained).
- If a TTA trocar was used then remove the catheter before removing the trocar sleeve to minimize contamination of the subcutaneous tissues. However, if a needle was used then the needle must be removed before the catheter in case the catheter is cut in half by the needle edge during withdrawal.
- Clearly this increases the risk of cellulitis and this author then instills 5-10ml of Crystapen solution into and around the stab incision. This author prefers to leave the stab wound unsutured.
- Complications include damage to tracheal rings during trocar insertion, cellulitis, emphysema, haematoma and loss of the catheter into the trachea.

Which Technique?

There are several advantages and disadvantages of each of the techniques to be considered when deciding which method (if any) to use.

Briefly and simply***, TTA and TW samples are best for bacteriology, BAL samples are best for cytology***.

There is a large variability in cellular content of TW samples and a large overlap between normal horses and those with lung disease. Hence TW cytology is often hard to interpret. However, in cases of localised lung disease (rare cases of pulmonary abscess/neoplasia) then the likelihood of 'missing' the specific affected bronchial segment with BAL makes TW the only useful technique. TW is useful as a quick and easy "screening" procedure – e.g. pre-competition – as a TW with a non-inflammatory cytological picture (e.g. only a few PMNs [<15%] and no eosinophils) is reassuring and prior sedation is unnecessary. However, where there is a prior suspicion of airway disease there is a strong argument for performing both TW and BAL – especially in a more mature horse (in contrast with a 2yo racehorse) where lower airway diseases such as RAO are more likely

BAL samples can be simply and effectively taken with minimal and relatively cheap specialised equipment (a BAL tube) and does not require an endoscope. BAL samples have a clearer normal cytological pattern leading to less interpretational problems. However, it is impossible to take a BAL sample without nasopharyngeal contamination and bacteriology on BAL samples is of highly dubious relevance. Additionally if sedation of the horse or interruption of an exercise programme are considered undesirable then TW is clearly preferable to BAL as the latter technique is very hard to perform in an unsedated horse.

As both techniques have their own strengths and weaknesses then it is preferable for both to be performed in order to maximise diagnostic information obtained during a respiratory investigation. With few exceptions, this author would generally submit TW samples for both cytology and bacteriology and BAL samples for cytology only.

Interpretation of Results

Analysis of TW and BAL samples is reasonably reliable at defining the presence or absence of significant airway disease (upper and/or lower airways) and is also useful for monitoring progress of a condition but it is less reliable at firmly identifying the underlying specific cause(s) of the airway disease. The main types of airway disease suitable for investigation using these techniques include RAO (classical stable-dust and summer-pasture associated disease), IAD, EIPH and primary bacterial airway disease.

Bacteriology

Interpretation of the relevance of bacterial growth in TTA/TW sample depends on the species cultured and the quantity of growth. A sparse or mild bacterial growth in TW fluid is generally not likely to be relevant to the disease process (unless clear respiratory pathogens are grown). There may be some bacteria present in a normal trachea in addition to possible mild contamination by oropharyngeal bacteria even during careful TW technique (look for oropharyngeal squamous cells as an indication of probable contamination). If quantitative bacteriology is performed then $>10^5$ colony forming units per ml is more likely to be relevant. A semiquantitative absent, sparse, mild, moderate, profuse grading of growth is also useful in this author's experience. However, it is important to interpret bacteriology in the light of concurrent cytology as even a profuse bacterial growth must be of dubious relevance (probable contaminant) 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 results in inevitable oropharyngeal contamination of the sample. Guidance on the likelihood of relevance of different bacterial species is listed in the table below.

Common Pathogens	***Streptococcus equi* ss *zooepidemicus*** ***Actinobacillus spp.*** ***Pasteurella spp***
Less Common Pathogens	***Escherichia coli*** ***Klebsiella pneumoniae*** ***Enterobacter spp*** ***Bordetella bronchiseptica*** ***Streptococcus pneumoniae*** ***Streptococcus dysgalactiae* ss *equisimilis*** ***Streptococcus equi* ss *equi*** ***Bacteroides spp*** ***Fusobacterium spp*** ***Peptostreptococcus spp*** ***Mycoplasma spp***
Likely Contaminants	***Pseudomonas aeruginosa*** ***Staphylococcus aureus*** ***Proteus spp***
Definite Contaminants	**coagulase negative *Staphylococci*** ***Bacillus spp*** ***Alternaria spp***

Table courtesy of Professor BruceMcGorum, Edinburgh University

Cytology

Cytocentrifugation followed by rapid air-drying (e.g. electric fan) results in best cell identification. Several choices of stain exist including "Diff-quik", May-Grunwald, Wright's-Giemsa, Leishman's, Toluidine blue (for mast cells) etc.. Magnification of 400X is usually adequate (up to 1000X oil immersion) and 200- 400 cells should be used for differential cell counts.

There is good correlation between BAL cytology and certain pathophysiologic measures such as exercised-induced hypoxaemia, lactic acidosis and airway obstruction as well as biopsy findings reinforcing the clinical relevance of this technique. BAL cytology often correlates poorly with TW cytology however.

TW generally has more neutrophils and less lymphocytes than BAL. The normal cellular constituents of TW and BAL fluid are shown below.

	TW	BAL
PMN (%)	<50	<10
LC (%)	<10	20-50
Macros (%)	40-80	40-80
Eos (%)	<2	<1
Mast cells (%)	<1	<2

Macrophages are the most abundant cells in both TW and BAL samples and are only of significance if they contain haemosiderin and hence indicate haemorrhage. Haemorrhage may have occurred at any site distal to the larynx and at any time from sampling to a few weeks prior to collection. The presence of a few haemosiderophages is normal in exercising horses and EIPH is therefore a clinical diagnosis.

Lymphocytes are more abundant in BAL fluid than TW. They may be difficult to differentiate from macrophages and it is often easier to consider mononuclear cells collectively. The precise significance of increased proportions of lymphocytes in either TW or BAL is undetermined though an association with increased proportions in BALF and some signs of respiratory disease has been identified. It has been suggested that airway lymphocytosis may be an indicator of chronic challenge by viral or other antigens.

By far the commonest abnormal finding in cytological analysis of airway secretions is **neutrophilia**. Neutrophil percentages are generally higher in TW than in BAL due to increased exposure to noxious stimuli that may result merely from housing or poor air hygiene. Interpretation is not only complicated by the effects of management but also by the wide range of reported results found in normal horses (3-83% neutrophils) and in horses with RAO (7-96% neutrophils). In horses with RAO there is no correlation between neutrophilia in TW and either BAL neutrophilia or histopathology. Whilst TW is of little use in the specific diagnosis of RAO it may be useful in the diagnosis of IAD in which a sub-section of cases show marked changes in TW but no changes in BAL. In TW samples, 20% PMNs is commonly used as a cut-off for inflammation based on studies in racehorses in which >80% of normal horses had <20% neutrophils and also based on associations with >20% neutrophils and coughing and other indicators of inflammation. The wide normal range for TW neutrophils makes this finding difficult to interpret and many normal horses in dusty environments show marked TW neutrophilias in the absence of disease. BAL is far more sensitive for detecting genuine airway neutrophilia due to the lower reference range. BAL neutrophils accounting for > 10-15% total cells confirm lung disease. Airway neutrophilia arises usually as a result of allergic lung disease (RAO, SPA-RAO), inflammatory airway disease (IAD) or infectious lung disease (bacterial or viral). These disease types cannot always be reliably differentiated simply on BAL although generally the magnitude of the neutrophilia is highest in RAO/SPARAO (e.g. often 30-70% PMNs and up to 90 %+) and also the results of TW culture and clinical signs and history add important differential diagnostic information. Neutrophilia in BALF is far more reliable in diagnosing distal airway inflammation because the effects of management (though still relevant) are less marked than in BALF and there is less variation between normal subjects. If BALF contains <5% neutrophils then one can be confident that there is no generalised distal airway disease. PMNs are far better preserved in RAO cases than in lower airway infections.

Occasionally increased numbers of eosinophils are seen for example in IAD, rare cases of lungworm or eosinophilic interstitial pneumonitis. Some young horses with 'Inflammatory Airway Disease' (IAD) may be found to have a high percentage of mast cells. Mast cells are almost exclusively seen in BAL. The presence of mast cells (much like eosinophils) is of undetermined significance. Whilst mast cells may be a finding in normal horses they have been associated with signs of respiratory disease and may represent very early RAO or a sub-section of IAD.

Repeated BAL within 48 hours of a previous BAL might detect a mild neutrophilia as a result of the previous wash but this is unlikely to present a significant clinical problem.