Ear Cytology

Correct identification of microbial pathogens in otitis externa is critical for treatment decisions and helps to promote good antimicrobial stewardship

Note: for successful resolution of otitis externa and to prevent recurrences, any predisposing, primary and perpetuating factors will also need to be identified and corrected.

Ear cytology can be simple, rapid and inexpensive, and samples are relatively easy to obtain from conscious dogs.

1 Restraining the patient

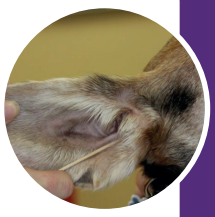
Step 1:

- **Ask an assistant to restrain the dog appropriately**
- **Sample the less severely affected ear first**

2 Sample collection

Step 2.1:

- **Insert a cotton bud gently into the vertical canal**
- **Rotate to collect material and withdraw from ear**



Step 2.2:

- **Roll onto a labelled glass slide**
- **Air dry for a few seconds** (heat fixing optional)

3 Staining

Before staining check under 4X for ear mites

Step 3:

- **Use a rapid Romanowsky type stain according to manufacturer's instructions (e.g. Diff-Quik®)**
- **Dip slide into each of the three solutions 5-7 times** (methanol fixative, eosin, methylene blue)
- **Rinse the underside of the slide under the tap**
- **Carefully blot off excess water using a clean tissue**

TOP TIP

Change solutions regularly depending on frequency of use (at least weekly when used every day). Different vials should be available for staining non-surface samples such as blood smears

4 Microscope examination

Step 4:

- **Examine under low power** (e.g. x4 lens) to locate suitable area of interest (single cell layer). Light intensity and condenser set low for this
- **Once in focus change to high power cytology settings without altering the level of the stage:**
  - **Rotate low power lens away**, apply a drop of immersion oil to the centre of the slide.
  - **Rotate the oil immersion lens directly into the oil.** Careful not to contaminate any other lens with oil!
  - **Increase light to highest intensity**
  - **Elevate condenser to (almost) highest position**
  - **Focus using the fine adjustment dial**

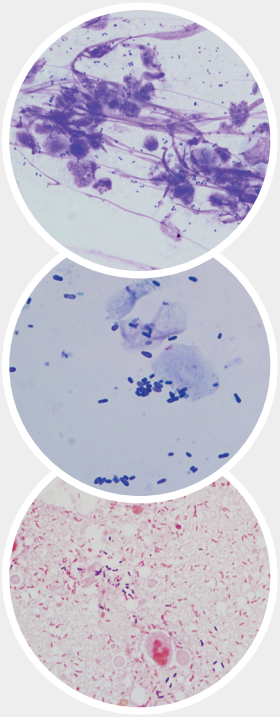
Microscope should be equipped with a good light source and high quality x100 oil immersion lens

What you might find under the microscope

Pathogens

The most common pathogens seen in microbial infections of the canine ear canal are recognisable as three different morphological types:

1. **Cocci:** most commonly *Staphylococcus pseudintermedius* (round blue spheres, usually in pairs or quartets). Less frequently *S. aureus, S. schleiferi*, coagulase-negative staphylococci, Streptococci, Enterococci. When in chains more likely to be Streptococci or Enterococci.
2. **Yeasts:** *Malassezia pachydermatis*: Easily identifiable due to characteristic budding shape ('footprints in the snow' or 'peanut' shape).
3. **Rods:** most often *Pseudomonas aeruginosa* (occasionally *Proteus mirabilis*, *Escherichia coli*), sausage shaped.



Inflammatory cells

Inflammatory cells that might be found are:

1. **Degenerate neutrophils** - (those with non lobulated nuclei) in association with bacterial phagocytosis confirms infection.
2. **Non degenerate neutrophils** - (those with lobulated nuclei) and no micro-organisms, are associated with a sterile process such as *Pemphigus foliaceus*.

Cytological examination of ear smears can be used to answer the following questions:

- Is microbial infection involved?
- Which type of microbes are present?
- If no microbes are seen, is there evidence of inflammation?
- Was treatment successful?

Every correctly obtained sample will yield some cells and ear canal debris. Even from healthy canals, non-nucleated epithelial cells and waxy debris of varying staining intensity should always be found (if not, repeat sample, review staining procedure and microscope set up).

Knowing what is normal and what is abnormal

Specific numbers of microbes per high power microscopic field needed to confirm a diagnosis of microbial infection have not been validated. Instead, a combination of clinical and cytological findings are used.

- Microbes in association with neutrophils means: infection highly likely – consider antimicrobial treatment.
- Infection unlikely if inflammatory cells seen but no microbes (send swab for culture to corroborate) – consider anti-inflammatory treatment.
- Cocci and yeast adherent to epithelial cells, may be normal for the individual or represent a microbial overgrowth – an ear cleaner may be all that is indicated.
- Few rods without inflammatory cells: likely contamination, commensal (eg *Corynebacterium* spp, gram positive rod, artifact).

Swabs submitted for bacterial culture are likely to yield bacterial growth even from healthy canals. Samples should be submitted when cytology reveals:

- Rods. Suspect *Pseudomonas* spp. Their presence suggests chronic disease. Investigation and elimination of other perpetuating causes (stenosis, otitis media) is advised.
- Cocci in a dog with a previous history of MRS infection, or if the regional prevalence of MRS is known to be high.
- If no microorganisms are seen despite neutrophilic/ pyogranulomatous inflammation (to rule out a sterile disease process).

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