**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.

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### 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)

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### 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

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### 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

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**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.

**Microscope should be equipped with a good light source and high quality x100 oil immersion lens**
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 (e.g. 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).