



Lifelong Ear Partnership

Veterinary Guidance: Fundamentals for
successful otitis externa treatment

How to perform ear cytology

Otic cytology is an inexpensive and quick test that can be undertaken with basic equipment. In this article we will detail techniques and basics of how to perform cytology. Cytology identifies the types of microbes present as well as the level of inflammation, information which is essential when selecting the correct treatment of an acute episode of otitis externa.



For a guide to the fundamentals of successful otitis externa treatment please review our previous article.

[Preparing an owner for](#)

[cytology](#)

Getting owners to understand why you are doing cytology will maximise compliance. Owner resources such as those on ear-inflammation.com can aid in getting owners invested in the journey. Links to explanatory videos such as <https://youtu.be/7J-VNI3CZsl> can be sent to owners over email or text when they book in for an otitis appointment.

When to perform cytology

Cytology should form part of the routine investigation of **every case of otitis externa**. It adds value in both acute and chronic cases. Furthermore, it can also be used for monitoring response to treatment, as we know, at each visit things can change microscopically that we may not otherwise be aware of! See figure 1 for a timeline of when to use cytology during investigation of an otitis externa case.

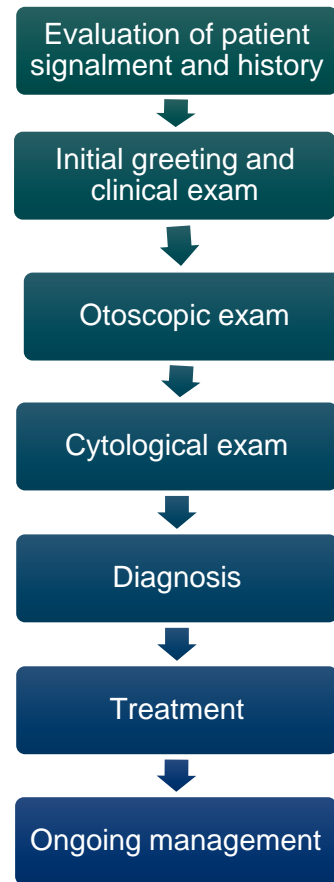


Figure 1 Timeline of Otitis Diagnosis and Treatment

Sampling technique

There are three commonly used sampling techniques in practice.

1. Cotton Bud Sampling
Allows sampling at the junction between horizontal and vertical canal (the preferred site for cytological sampling).
2. Gloved Finger Sampling
A good choice when the ear is painful, and sampling is challenging.
3. Cytology Brush Sampling
Unlike cotton buds, cytology brushes don't absorb any material.

Preparation of samples

- 1) Samples should always be taken from **both ears**, for cases of unilateral otitis it allows for comparison of the affected ear to the healthy ear, for bilateral otitis it alerts you to differences in each ear which may require different treatment.
- 2) Gently apply a thin layer of material onto the slide
- 3) Too much pressure can cause cell damage.
- 4) Ensure to label your slides correctly using a pencil – you can easily fit both ears on one slide to save time and slides
- 5) In cases where you suspect parasites start by examining an unstained sample on low magnitude

Sample fixation

For any sample which has a ceruminous / waxy / greasy component we want to fix the slide to prevent the material falling off when stained. Use a gentle heat source such as a hair dryer. Beware alcohol fixative tends to dissolve ceruminous material from otic samples; therefore it is best to avoid this stage and fix the sample with heat instead. However, if the sample is purulent or there is only minimal ceruminous material alcohol fixing would still be recommended.

Sample staining

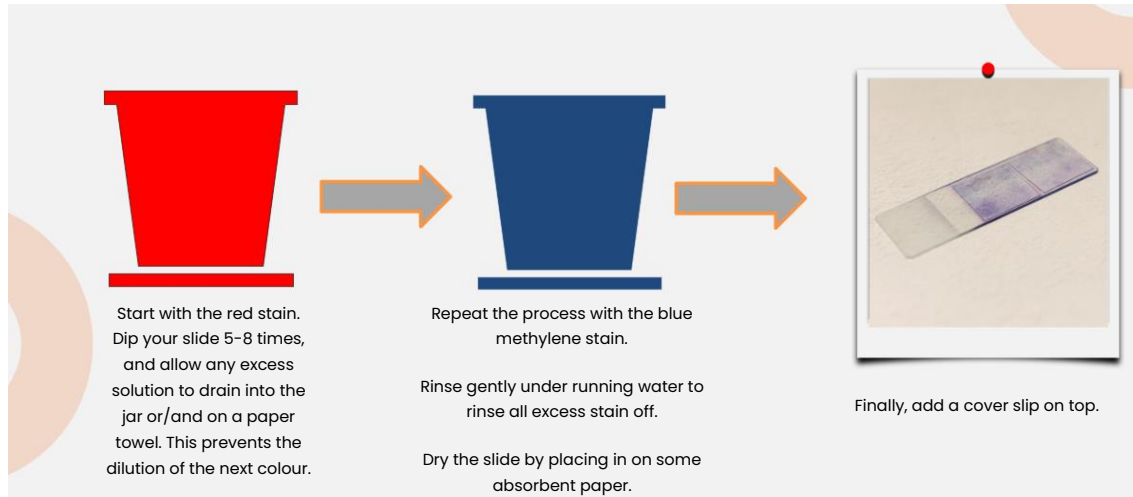


Figure 2: How to stain slides using DiffQuik

See figure 2 for a description of the staining process using DiffQuik.

Dechra's Tips for Cytology Staining

It is difficult to overstain the eosin (red) portion so if you are unsure make sure you dip the slide a few extra times.

- Microbes can grow in the stain solutions which can lead to false results – make sure the solutions in the pots are changed regularly. If you are concerned about wasting unused stain, use smaller jars that are just large enough to fit one slide in.
- If samples are staining poorly, replace the stain.
- Keep lids closed on the jars to prevent the solutions evaporating, jars with twistable lids are preferable.
- If you are short on time and not concerned about cell morphology you are able to get an overview of microbial overgrowth using just the blue stain on its own.
- Remember to always follow the instructions provided by your stain manufacturer as specific advice can vary.
- The slide can now be examined under the microscope. See figure 3 for a guide to using the microscope.

- In the next article we will cover common things seen under the microscope.

FOUR

Look into the eye pieces and adjust the distance between them until the right and left images merge into one. You may need to rotate the eyepieces themselves to adjust the focus for your eyes

THREE

Place the slide on the stage with the material facing upwards and turn the lowest powered objective lens into place first.

SIX

Finally, move the low powered lens(es) out of the way and add a drop of immersion oil. Go round to the oil immersion objective (x100), turn up the light source and slowly adjust the fine focus wheel to visualise the detail. The oil immersion lens will allow you to determine different cell types, and confirm the presence of coccoid vs rod bacteria

TWO

Ensure the illumination is on the light beam diaphragm is open and set the light to a comfortable (low to medium) setting. The light does not need to be at the maximum level to begin with.

FIVE

Scan the slide on the low powered lens (usually x4) using the course focus wheel to adjust the focus and assess for parasites. Then find an interesting area to examine in more detail.

If your microscope has further lenses (x10 for example) then you could consider reviewing the slide using these lenses to determine areas of interest to examine in high power.

The high powered dry lens (usually x40) can be used to visualise yeast and bacteria and to give an idea of quantification of organisms.

ONE

Turn the microscope on at the wall and on the power switch

