Cytology (and Microscopy) IN DERMATOLOGY
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Introduction

Cytology is defined as the collection of cellular material and fluid for microscopic examination. This procedure is heavily used in all aspects of medicine, but is of tremendous value in dermatology cases. The skin is an external organ, and thus, the lesions we must evaluate are readily accessible for this diagnostic procedure. It would be a great waste NOT to perform cytology on our cases. In fact, it is indicated in almost all patients presenting with skin disease.

Cytology provides the veterinarian with useful information about the etiology, pathogenesis, and severity of skin diseases. Cytologic techniques are very useful as a monitoring tool in dermatology cases, for example to monitor the presence of infectious agents in the skin or ears following therapy. Cytologic evaluations also Lastly, cytology is a cost effective diagnostic procedure. It will generate income for a veterinary practice (while providing essential information for managing patients.

There are three important aspects of these tests: 1) selection of the proper lesions or locations from which to collect samples, 2) proper collection of materials, and 3) interpretation of the material. We will focus on selection of lesions and proper collection techniques for cytology.

Indications

Cytology is indicated in ALL dermatology cases. It is most helpful in determining the cause of pustules, papules, nodules, tumors, draining tracts, chronic ulcerations, or plaques. When examining the sample we are specifically looking for:

1. The types and numbers (relative or absolute numbers) of cells. By examining cells we determine if there is inflammation, and if inflammatory cells are present, their type and number. We also are evaluating cells for criteria of malignancy.

2. Types and numbers (subjective) of infectious agents. Here, we are looking for the presence of infectious agents, primarily bacteria and fungi (including yeast). The types of organisms, their presence within inflammatory cells, the presence of a single population vs a mixed population is noted, and relative numbers are recorded.

Materials & Methods / Techniques

For the most part, cytology is a very cost effective diagnostic procedure. Other than a quality microscope, the only supplies required are swabs, syringes, needles, glass slides, a camel hair brush (wait and see!), and various stains. Several stains are available for cytology. Romanowsky stains such as Wright's stain, and modified-Wright's stains like Diff-Quick Stain® are easily and quickly performed. They are excellent when permanent slides are desired. Supravital stains such as New Methylene Blue are also easy and rapid. Each clinician should choose a stain that he/she is comfortable with and become accustomed to its staining qualities.

Several techniques may be used to obtain samples: 1) Fine-needle aspirate; 2) Impression smears made directly from the surface of cutaneous lesions & impression smears made from cut surfaces of lesions (e.g., tumors) removed from the skin; 3) Scrapings of tumors or nodules; 4) Lancing pustules or papules to remove contents for examination; and 5) Swabs made using cotton-tipped applicators. Each technique has advantages and is best suited for specific lesions.

Samples may be distributed on a slide by non-traumatic imprints, "squash" preparations or brush cytology. In the "squash" technique, the sample is placed on the slide, and then another slide is placed over the slide with the sample. No pressure should be placed on the sample! The top slide is then gently pulled away from the slide with the sample, leaving the specimen distributed across the slide.

In brush cytology, the sample is placed on a clean glass slide and then spread out using a camel hair brush or nylon artists brush. The brush is then rinsed well with tap water prior to each use and after each use to prevent contamination. When performed properly, this technique gives single cell layer distribution on the slide, with a minimum of trauma to the sample.

Slides should then be fixed and stained. One slide should be left unstained in case a special stain is needed. There are multiple options for staining cytologic specimens. Most clinicians prefer a Romanovsky type stain (Wright's stain, Giemsa stain, or modified Wright-Giemsa [Diff-Quik] stain). New Methylene Blue stain is a supravital stain that is especially good at providing nuclear detail.

Slide Examination
The entire slide should first be examined under a low (scanning) power. On most microscopes the lowest power is 40X (a 4 power objective), however, lower power objectives (e.g., 2X) are available. The scan is performed to evaluate the staining of the slide, to identify areas that should be examined more closely, and to identify large structures (e.g., foreign bodies, hyphae, & Demodex mites) that may be missed under higher power. After the scan is completed, the slide should be examined using low power (10X objective) and oil immersion (50-100X) objectives.

Tip 1: Always keep one hand on the fine focus knob while scanning a slide. You should be slowly moving that knob back and forth, adjusting the focal plane to allow you to see materials at different depths of the slide. This is especially critical on cutaneous impressions and slides when the material is somewhat thick.

Tip 2: Most microscopes have a "high dry" objective, usually a 40X objective giving a total magnification of 400X. These objectives are designed to work best when the slide has a cover slip. Otherwise the image will be slightly blurred. So,...place a drop of immersion oil on the sample, then add a cover slip, if you use this objective....you'll be impressed with the difference.

Submitting Samples for a Second Opinion
It is often useful to have the slide evaluated by another individual and/or to send the sample off for evaluation by a specialist in cytology. In general, it is recommended to contact the individual to whom you are intending to send the material, and ask their preferred methods of handling and submission. Most cytologist would like to receive 1-2 unstained, unfixed slides, and 2-3 Romanovsky-stained slides for their review. Some prefer to have the specimen (aspirate or fluid) sent in an EDTA blood collection tube, as well. Fluid specimens should have slides made as soon as possible for examination, even if the fluid is to be sent away for evaluation.

Slides may be placed in a plastic or cardboard slide mailer, taped closed, and wrapped in bubble wrap or a padded envelope for mailing. The package should be labeled as "fragile" and/or as "glass". It is always important to include a brief history of the problem, a drawing indicating the source and location of the lesions, and other appropriate data. Remember, cytology is only one piece of the puzzle.
Ear Cytology

Cytology of debris collected from the external ear canal is a useful and valuable technique to assess current status and monitor response to therapy. Cytology of material collected from the ear should be performed every time a patient is examined for an ear problem. Materials required for this procedure include cotton swabs, glass microscope slide, matches, Diff Quik7 stain, and a microscope.

To collect representative material from the ear, a dry cotton swab is inserted into the external ear canal and advanced slowly and gently. The goal is to reach the horizontal component of the ear canal. This is made easier by grasping the pinna and gently pulling the ear out and downward. The swab is slowly rotated during the process to help collect debris and exudate. The swab is then rolled out on a slide. TIP: I always examine both ear canals. I will place samples from both ears on the same slide, material from each side on opposite halves of the slide. This saves time when examining the slide.

The slide is heat fixed by holding a match or lighter under the slide for 2-3 seconds. The slide should come warm, not hot! TIP: All slides that have greasy materials on them should be heat fixed to help adhere the material to the slide. Slides should then be stained using the preferred stain. Note that even with heat-fixation, much of the debris will be lost during the staining process. Therefore, if Diff-Quik stain is used, the process must be very, very gentle. Also, debris and infectious agents will contaminate the stain. It is necessary to change stain regularly (at least weekly) to prevent misinterpretation of results. After staining, examine the slide under oil immersion for the presence of bacteria (rods and cocci) and yeast (Malassezia sp.).

Bacteria are only occasionally seen in normal ears; Malassezia are occasionally seen as well (1-2/oil field is normal). Epithelial cells are usually seen in small numbers. The relative number of bacteria, yeast and epithelial cells should be recorded using an arbitrary scale (i.e. 1-4+). The presence and relative number of neutrophils should also be noted. Neutrophils without bacteria suggest a hypersensitivity reaction to medication being placed in the ear (neomycin and propylene glycol being the biggest offenders).

Making the Most of Your Microscope

Microscopes are indispensable instruments for a veterinary practice. If you’ve graduated more than 5 years ago….microscopes have changed. They have better optics, are more ergodynamic, and in many cases, are less costly for a higher quality scope. When purchasing a microscope, consider one that is double-headed, which allows two viewers at one time. This is very helpful for quality control….an absolutely necessary aspect for you, other veterinarians in the practice, and your technicians! A camera that allows viewing on a monitor is also helpful, but the resolution is never as good as looking directly through the scope. The monitor system does allow for excellent client education. We frequently bring clients back to our lab to see parasites (doesn’t everyone?), bacteria, neoplastic cells, yeast, and other obvious microscope items of interest. Client compliance….and willingness to proceed with other tests or treatment…tends to increase dramatically once the client has “seen” what is going on with their pet.

General rules in microscopy:
1. Keep the scope clean. Twice daily cleaning by a veterinary technician is ideal..along with cleaning whenever the scope is used. In addition, it is helpful to have the scope professionally cleaned and lubricated 1-2 times yearly. It will make a huge difference in the functionality of your instrument.
2. Keep the scope covered when not in use. All hospitals tend to be dusty and have hair floating about….which can damage the scope.
3. Use a different microscope for fecal examinations. Fecal solutions (sugers, salt solutions) are quite caustic if they come in contact with the microscope lens or get “spilled” onto the slide platform.
4. Adjust the scope (at least once daily) for Kohler illumination. This will help to “focus” your scope for use.

Summary

Cytology is one of the most useful diagnostic tools for dermatology. Virtually every dermatology case presents with multiple opportunities for cytologic evaluation….and the information that cytology provides, is often the key diagnostic information that marks the therapeutic path for that patient.
Table 1. Indications for Various Cytological Collection Methods

<table>
<thead>
<tr>
<th>Technique</th>
<th>Ideal Lesions</th>
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<tbody>
<tr>
<td>Fine-needle aspiration</td>
<td>Nodules</td>
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<tr>
<td></td>
<td>Tumors</td>
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<tr>
<td>Direct smears (imprints)</td>
<td>Plaques (e.g., eosinophilic plaques)</td>
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<td></td>
<td>Ulcers</td>
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<tr>
<td></td>
<td>Crusts (after removal of crust)</td>
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<td></td>
<td>Cut surfaces of tumors, nodules, etc. after removal</td>
</tr>
<tr>
<td>Scrapings</td>
<td>Tumors, nodules, etc. after removal</td>
</tr>
<tr>
<td></td>
<td>Scales / crusts</td>
</tr>
<tr>
<td>Lanced lesions</td>
<td>Pustules</td>
</tr>
<tr>
<td>Swabs</td>
<td>Draining tracts</td>
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**Tuning the Microscope for Maximum Clarity (Köhler Illumination)**

*Note:* *Tune the microscope with the 10 power objective down in position*

1. Focus on any slide (cytology or histopathology)
2. Lower the condenser stage as low as it will go. This is done using the knob on the left side of the microscope.
3. Close the (aperture) diaphragm in the condenser AND the (field) diaphragm aperture on the base of the microscope. You should now see a circle of light in the visual field.
4. If your microscope has a lens in the condenser that can be up or down….flip it up. Some microscopes have a cone-shaped condenser that does not have a moveable lens. In that case, close the (condenser) aperture as far as possible.

Raise the condenser stage (while watching the visual field) until the circle of light is at its smallest and has sharp borders.

5. Center the circle of light using the set screws on the condenser stage (at the 4 and 8 o'clock positions)…there are usually two set screws that move the condenser.
6. Open the field diaphragm (on microscope base) until the light fills the entire visual field.
7. Open the condenser aperture ring (on the condenser) according to the objective you are using. For lower objectives (2 and 4 power) the ring should be set on approximately the .2 position. For higher objectives, the ring is set higher (0.4 for the 10 power; 0.6 for the 40 power : 0.6-0.8 for the oil immersion 100 power objective)
Tip: To increase contrast (e.g., to help visualize eosinophil granules or parasites) close the condenser aperture ring or lower the entire condenser stage.

*Courtesy of Dr. Jim Noxon, Iowa State University.*