Introduction
Cytology is defined as the collection of cellular material and fluid for microscopic examination. This diagnostic procedure is heavily used in all aspects of medicine, but is of tremendous value in dermatology cases because the skin is an external organ, and the lesions we evaluate are readily accessible for this diagnostic procedure. In clinical practice, cytology is indicated and very helpful in managing almost all patients presenting with skin disease.

Cytology provides the veterinarian with useful information about the etiology, pathogenesis, and severity of skin diseases. Cytology is a very useful as a monitoring tool in dermatology cases, to help evaluate therapies by monitoring the presence of infectious agents in the skin or ears. Lastly, cytology is a cost effective diagnostic procedure and 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.
Several techniques may be used to obtain samples: 1) Fine-needle aspiration; 2) Impression smears made from the surface of cutaneous lesions, from under crusts & 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) The use of cotton-tipped applicator swabs to “roll” in difficult-to-reach areas (e.g., facial folds). 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 small camel-hair brush or nylon brush. The brush is rinsed well with tap water prior to each use and after each use to prevent contamination. When performed properly, this technique gives excellent sample distribution on the slide with a minimum of trauma to the cells.

After the slide is prepared, the sample should be fixed and stained. In some cases, such as for unusual lesions or lesions that have failed to respond to previous treatments, it is helpful to make an extra slide and leave it unstained. That slide may be stained later with a special stain (e.g., Gram’s stain) or sent to a consultant to be stained at their discretion. There are multiple options for staining cytologic specimens. Romanowsky stains such as Wright's stain, and modified-Wright's stains like Diff-Quick Stain® are easily and quickly performed. When processed appropriately by adding a cover slip, these slides may be saved indefinitely and can serve as a “library” of slides for future review and reference. Supravital stains such as New Methylene Blue are also easy and rapid, but are more difficult to preserve or mail out for a second opinion.

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.

Tip 3: Increase refractivity to highlight parasites, foreign bodies, or (eosinophilic) granules by either 1) lowering the condenser stage or 2) closing the aperture diaphragm. This will increase
“glare” and allow light to bounce off of these structures and make them easier to see. Really.

Interpretation of Cytology
When examining any cytologic preparation, we are looking to see: 1) if inflammation is present, and if so, what are the cell types involved, 2) are parasites or micro-organisms present, and if so…what types and relative numbers are present, 3) what cells are present and do they exhibit normal characteristics of cells found in the lesion sampled (look for atypia, clumping of cells, any characteristics of neoplasia, etc.). The microscopic findings on the slide are always interpreted in light of the clinical findings.

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 cytologists 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 quickly to avoid evaporation and residue formation.

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". Slides for cytological evaluation should not be sent in the same mailing package as formalin, which may alter some cellular characteristics desired for cytology. 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.

The Microscope
Microscopes are indispensable instruments for a veterinary practice. When purchasing a microscope, consider buying one that is double-headed, which allows two viewers to see the images 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. However, 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 (sugars, 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.

References available upon request.

Tuning the Microscope for Maximum Clarity (Kohler Illumination)

The purpose of “tuning” the scope to get all of the components (especially the various lens and prisms) into as perfect alignment as possible. This will minimize the diffraction (scatter) of light that reduces image quality. Ideally, the tuning process should be done separately for each objective, but that would become tedious if done every time you changed objectives. Performing this adjustment one time, as you start your cytology examination, using the 10x objective (selected because it is easy and normally our starting point for the cytological examination) works well in actual practice.

Step-by step tuning
1. Select the 10 power objective
2. Focus on a slide (cytology or histopathology) to give a baseline image
3. Lower the condenser/stage to the lowest point of adjustment (i.e., bottom)
4. Close the aperture (field diaphragm) on the base of the microscope and the condenser aperture. You should now see a small circle of light or fuzzy darkness in the visual field.
5. Now, raise the condenser stage (while watching through the scope) until the circle of light is at its smallest and has sharp borders. This will be when the condenser is near the top.
6. 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 (sometimes adjustable with an Allen wrench) that move the condenser.
7. Open up the aperture (field diaphragm) on the base of the microscope until the circle of light just barely fills the visual field.
8. Adjust 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)
9. To increase contrast (e.g., to help visualize eosinophil granules or parasites) close the condenser aperture diaphragm or lower the condenser.