Second Edition

Clinical Atlas of Small Animal Cytology and Hematology

Andrew G. Burton

WILEY Blackwell

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Second Edition

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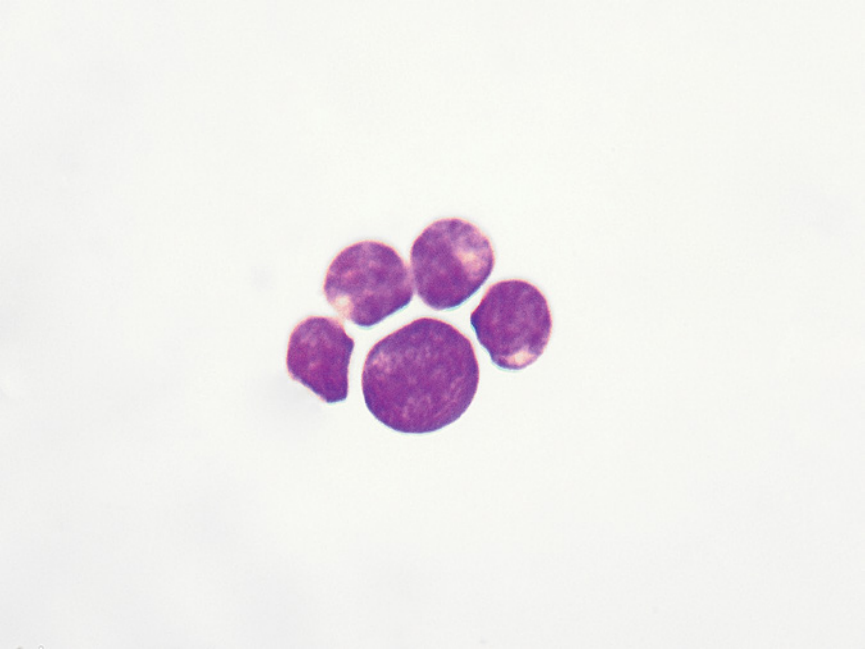
Dedication

To the veterinarians, technicians, and veterinary professionals who dedicate so much of themselves to the care, health, and well-being of animals. Your passion and sacrifices are seen, and so deeply appreciated.



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Preface

The *Clinical Atlas of Small Animal Cytology and Hematology* returns, with brand new hematology chapters and fully updated cytology sections. More comprehensive than ever, the mission of this textbook remains unchanged: to empower veterinary medical professionals with increased knowledge and confidence in cytology and hematology through exceptional images, a guided approach to interpretation, and succinct yet thorough supporting text.

Images form the keystone of this atlas, and this extensive collection is unparalleled for the size, clarity, completeness, and representative nature of the photomicrographs. The images have been carefully curated to mimic the experience of microscopy and highlight the important diagnostic features that lead to the confident interpretation of samples. The power of these uniquely large images is perhaps best highlighted in the new hematology chapters, where they readily showcase the frequently small cells, infectious agents, and inclusions present in the blood.

Multiple examples of common conditions are provided to highlight the exciting (though often challenging) variations that may be encountered in practice. Additionally, images of different cells that may be confused with each other have been thoughtfully arranged for easy side-by-side comparison. Photomicrographs are accompanied by detailed descriptions, figure legends and annotated with arrows when needed to guide readers through the diagnostic process and ensure all important elements of samples are appreciated. Chapter 2 provides a guided approach to the interpretation of cytology samples, including cell types, criteria of malignancy, and common artifacts. This atlas contains only images of conditions where the diagnosis was confirmed by histopathology, special stains, infectious disease testing, pathognomonic cytologic features, or other confirmatory tests. All samples are stained with Romanowsky stains unless otherwise specified.

An in-depth hematology section is an exciting new addition to this textbook. Chapter 16 provides detailed instructions to prepare and stain high-quality blood smears, and a step-by-step guide that can be used at the microscope for a complete evaluation of the smear. Common hematology procedures are also described, with easy-to-follow instructions. Subsequent chapters in the hematology section provide a comprehensive catalog of cells, inclusions, infectious agents, and other components seen in blood, both common and rare, accompanied by interpretive guides, helpful hints, and fully referenced text.

The cytology chapters have been fully revised, and all contain new conditions and images. A new chapter detailing sample acquisition, preparation, and staining opens the textbook to help ensure the creation of the highest quality samples – important for viewing in-house, with point-of-care analyzers, or submission to the laboratory. Our knowledge of the complex world of pathology is constantly changing, and this edition has been critically referenced with the most current, relevant, and scientifically robust studies to aide in optimal, evidence-based decision-making. This edition retains the popular easy-to-read bullet point format, which increases efficiency without compromising the completeness or scientific rigor of the information.

From practitioners to pathologists, trainees to technicians, and every professional that makes veterinary medicine great, may the *Clinical Atlas of Small Animal Cytology and Hematology* be a powerful resource to help in our collective endeavor for the best possible patient care.

Acknowledgments

My sincerest gratitude to Wiley Publishers and the incredible team that continues to believe in the vision and impact of this book.

Thank you to the veterinary community for your support. You inspire this textbook and my work as a pathologist.

I would like to acknowledge the unwavering and generous support of my colleagues – most notably Cathy Greene, Dr. Maria Vandis, Dr. Raquel Walton, Dr. Karl Jandrey, and Dr. Bill Vernau.

And with all my heart, thank you to my family, especially Dr. Eric Franson – your love is the fuel for it all.

1

Cytology Sampling and Preparation

1.1 Cytology

Cytology is a useful, rapid, noninvasive, and safe diagnostic procedure with often strong correlation to histopathology, including high specificity for the diagnosis of neoplasia in cutaneous and subcutaneous masses [1, 2], lymph nodes [3], and oral tumors [4] in dogs and cats as well as bacterial infection in fluid samples [5]. Importantly, the quality of the sample, especially cellularity, has a significant effect on the interpretation and accuracy of results [6, 7].

Quality of cytology samples depends on four major factors:

1) Sample collection.
2) Slide preparation.
3) Staining.
4) Sample handling.

The ideal sample is adequately cellular, distributed in a monolayer, free of artifacts or contaminants, and is well stained. Point-of-care options for cytology have increased dramatically in recent years, further necessitating the need for clinics to be confident and proficient in the preparation and staining of high-quality cytology samples. This chapter provides a detailed description of how to collect, prepare, stain, and handle cytology samples. Following these steps will increase the chance of creating excellent quality cytology samples that maximize accurate interpretation, which is described in Chapter 2.

1.2 Sample Collection and Preparation

1.2.1 Selecting Lesions for Cytology

Not all masses or lesions are amenable to cytologic evaluation, and thoughtful selection of suitable lesions for cytology is an important component of obtaining diagnostic samples. Highly vascular lesions may yield only blood, while some mesenchymal lesions exfoliate cells poorly for cytology. It is also important to be mindful of the limitations of cytology. Only a small portion of the lesion or organ of interest is sampled, and the limited number of cells available may not represent the underlying pathologic process. Additionally, cytology cannot evaluate tissue architecture such as tissue or vessel invasion, sometimes limiting the evaluation of biologic behavior, which does not always correlate with cytomorphology. Specificity is usually higher than sensitivity when comparing cytologic and histopathologic interpretations, which is often directly related to the aforementioned limitations of reviewing only a small number of cells from a focal area of a lesion or an organ [2]. Agreement between cytology and histopathology can also be low due to limitations of focal sampling and lack of tissue architecture; particularly relevant when evaluating lymph nodes for metastatic disease [3, 8].

1.2.2 Preparing the Site

Special preparation of the site (e.g., clipping of surrounding fur or sterile preparation of the skin) is not required for most lesions. If excessive surface debris, dirt, or gel (from topical medications or ultrasound procedures) is present, this may be cleaned with sterile saline or alcohol; however, ensure that the area is completely dry prior to sampling. For sampling of internal organs, the skin should be clipped and surgically prepared prior to the procedure.

1.2.3 Sampling Techniques and Slide Preparation

Cytology samples may be obtained by numerous techniques including needle collection, swabs, scrapes, impressions, and brushes, which can be tailored to the nature and accessibility of the lesion, organ, or region of interest. Needle and impression sampling are best performed in areas free of ulceration or tissue necrosis, and it may be helpful to sample from multiple areas, including the center

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and edges of the lesions as well as deep and superficial regions to increase the probability of a diagnostic sample. If possible, multiple slides should be made to increase cell yield and the likelihood of diagnostic samples, and using different techniques to make multiple slides may be helpful. The following sections will describe common sampling techniques in more detail.

1.2.3.1 Needle Collection

Needle collection (with or without syringe aspiration) is the most common and usually the most effective technique to obtain diagnostic cytology samples, as it can collect cells from different areas and depths of the lesion. It is commonly used to sample cells from skin masses, lymph nodes, and internal organs.

Supplies Slides must be made immediately after sample collection to avoid clotting or drying of the aspirated material, so it is important to have all supplies ready prior to sampling. Supplies needed include the following:

- Latex or nitrile gloves.
- Glass microscope slides with a frosted edge.
- A pencil.
- Sterile needles (20, 22, or 25 gauge).
- Syringes (3, 6, or 12 ml).

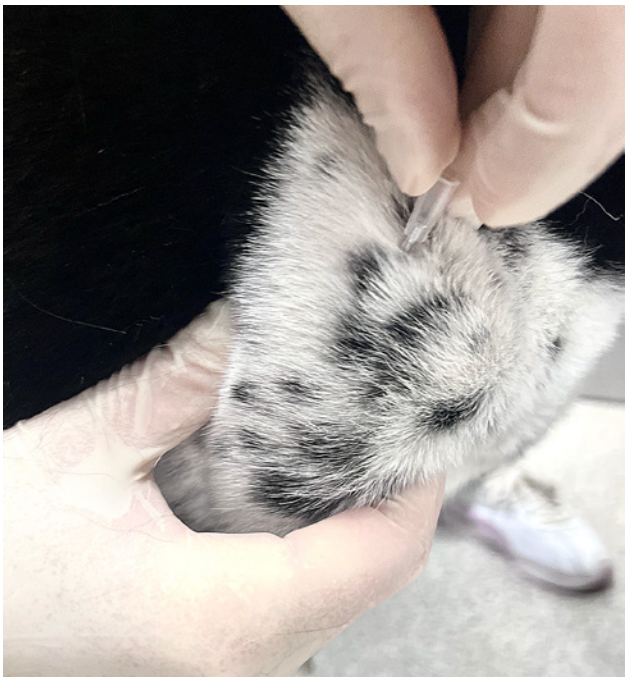


Figure 1.1 Non-aspiration needle collection technique. Note that the mass is stabilized in one hand, while the other hand directs the needle into the mass in short, sharp movements through different planes without leaving the skin. Courtesy of Dr. Eric Franson.

Procedure

1) Select appropriate needle.
   Starting with a 22-gauge needle is recommended. In one study, there was no difference in cellularity when using 22- and 25-gauge needles; however, 25-gauge needles were associated with greater cellular trauma, while 22-gauge needles had greater blood contamination [9]. Importantly, there was no difference between the needle gauges in the ability to make a diagnosis. A 20-gauge needle may sometimes be appropriate (e.g., for some mesenchymal lesions, and bone lesions); however, blood contamination may be an issue.
2) Start with a non-aspiration technique.
   For most masses and lymph nodes, it is recommended to start by using just the needle. Stabilize the mass or lymph node as appropriate/possible with one hand and hold the hub of the needle in the dominant hand (Figure 1.1). Direct the needle into the mass and collect material using short, sharp movements, redirecting within the mass in multiple different planes without leaving the skin. Typically, 3–5 redirections are sufficient to collect adequate material. *Note*: Material may not be visible within the needle, even if an adequate sample has been collected. If blood is seen in the needle, stop the procedure immediately and make slides with that sample. Resampling with a smaller needle gauge or less redirection of the needle should be attempted to obtain samples with no or minimal blood contamination.
3) Add aspiration if needed.
   If no material is retrieved, or cellularity is very low, applying negative pressure using a syringe attached to the needle during collection may increase cellular yield (Figure 1.2). This technique can increase blood contamination and may result in cell damage or rupture, especially if using large syringes or if excessive pressure is applied. In one study investigating lymph node aspiration, there was no difference in sample quality (including cellularity, blood contamination, and cell preservation) between aspiration and non-aspiration techniques [10]. *Note*: It is important that no aspiration or negative pressure is applied when removing the needle and syringe from the lesion, as this can disperse the sampled material irretrievably throughout the syringe.

Internal organs are sampled using needle collection and is ideally performed with ultrasound guidance. Longer spinal needles are usually required to reach the organ of interest. For highly vascular organs, including the spleen and liver, the needle-only technique may be helpful.

Figure 1.2 Aspiration needle collection technique. A syringe can be attached to the needle and used to apply negative pressure to help increase cellular yield in poorly exfoliating lesions. Courtesy of Dr. Eric Franson.

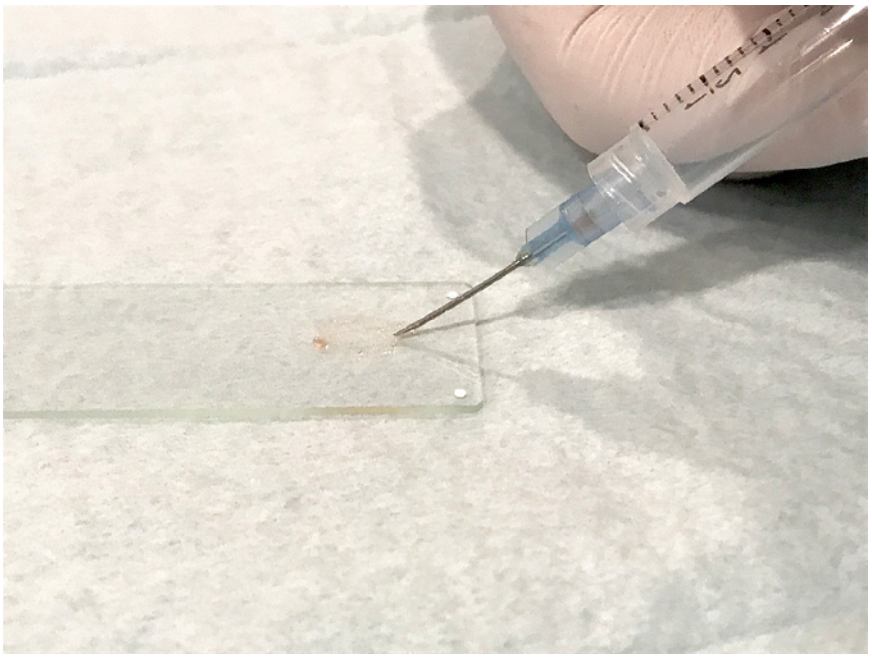
Conversely, for intra-abdominal lymph nodes, the aspiration technique has been shown to provide higher cellularity and was more likely to be diagnostic than non-aspiration techniques [11].

Fluid-filled lesions are also best sampled with a needle aspiration technique. The aspirated fluid can be used to make slides, and any additional fluid can be stored in EDTA tubes to make additional slides at a later time, or in plain tubes for microbial culture and susceptibility testing if desired. When fluid is drained from some lesions, a solid component of the mass may become apparent, and sampling of this region can be fruitful to further evaluate the mass or underlying process that may be causing the fluid accumulation.

Slide Preparation It is important that slides be made immediately after collection of material, as any delay risks clotting or coagulation of the sample or desiccation of cells.

1) Lay multiple, clean slides on a solid, flat surface and label those by writing patient details on the frosted edge of the slide with a pencil.
2) Attach an air-filled syringe (typically 6 or 12 ml) to the needle hub (this can be the same syringe if the aspiration technique was used).
3) Touch the needle to the glass slide near the frosted edge with the bevel facing downward (Figure 1.3). Touching the glass surface will help prevent material spraying over a large distance and trauma to the cells.
4) Depress the syringe with gentle force, using air pressure to expel a small amount of the contents from the needle hub. *Note*: Greater pressure can always be used to expel more material if needed, so start with gentle force. Repeat this step on multiple slides if enough material is present in the needle hub.

Figure 1.3 Transferring the sample to glass slides. Note that the bevel of the needle is facing downward and is touching the glass slide to ensure the sample remains in a focal area for optimal spreading.



5) Rest a clean glass slide on top of the slide with the material (Figure 1.4). Using only the weight of the top slide, with no or minimal downward pressure, pull this along the lower slide to spread the material in a monolayer (Figure 1.5). *Note*: Do not use the needle to disperse the material on the slide. This often results in trauma to the cells and thick linear aggregates of material that are difficult to evaluate (Figure 1.6).
6) Air-dry the slides by blowing on them or using the cool setting on a hair dryer [12].

Slides should be left for an additional 1–2 minutes prior to staining to ensure they are completely dry. *Note*: Slides should *never* be heat fixed. Heat fixing does not improve cellular yield, including for bacteria and infectious agents, and may cause heat damage to cells [13, 14].

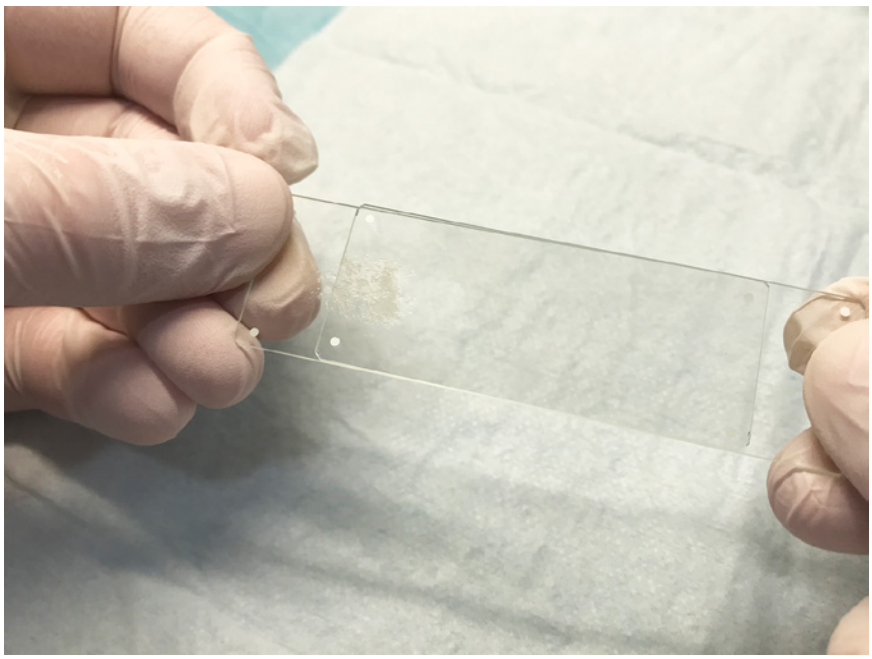


Figure 1.4 Spreading the sample. Rest a clean glass slide on top of the slide with the sample and allow the sample to spread.

Figure 1.5 Spreading the sample. Use only the weight of the top slide, with no or minimal additional downward pressure to spread the sample in a monolayer across the slide.

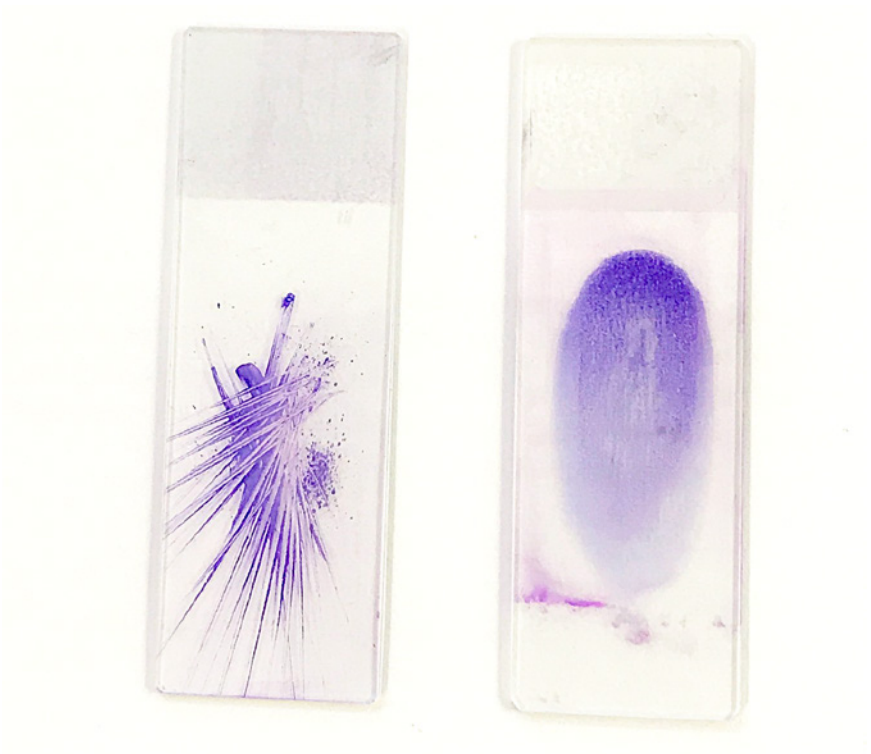


Figure 1.6 An ideal cytology slide is present on the right, with a monolayer of cells. A needle was used to disperse the material on the slide on the left, which can rupture cells, and create linear areas that are too thick for cell evaluation.

For fluid samples, a direct smear should always be made at the time of sampling, either using the technique described earlier or using a blood smear technique (see Chapter 16). This is essential to appreciate the cellularity and distribution of cells. Concentrated samples may also be created by spinning down the fluid in a centrifuge, removing two-thirds of the supernatant, and reconstituting the concentrated cell pellet within the remaining fluid and then making the slide as described. Squash preparations of any chunks of tissue or material may also be helpful to increase cellularity, especially for bronchoalveolar lavage (BAL) or transtracheal wash (TTW) samples.

1.2.3.2 Swab Sampling

Swabs are most commonly used for sampling from ears, vaginal cytology, and exuding lesions including fistulous tracts or nasal discharge.

Supplies Similar to needle aspirates, slides must be made immediately after sample collection to avoid drying of the aspirated material, so it is important to have all supplies ready prior to sampling. Supplies needed include the following:

- Latex or nitrile gloves.
- Glass microscope slides with a frosted edge.
- A pencil.
- Sterile cotton swab.
- ± Sterile water.

Procedure Introduce the cotton swab into the area of interest, being careful to avoid contamination with surrounding tissue (e.g., the outer pinna for ear canal swabs or the vestibule for vaginal cytology samples) (Figure 1.7). If the area is dry or crusted, the tip of the cotton swab may be moistened with sterile saline to aid in collection, cell yield, and sample preparation. The swab may be spun or gently pressed against the lesion or tissues to collect material.

Slide Preparation For swabs, roll the samples with gentle pressure to create a thin, linear row of material along the slide (Figure 1.8). Do not wipe or use excessive downward pressure, as this may rupture or damage cells.

1.2.3.3 Scrapes

Scrapes are most commonly used to evaluate for ectoparasites, but may also be useful for dry, flat, or crusted skin lesions, including those associated with other infectious agents including dermatophytes [15]. They may also be used to collect cells from masses removed for histopathology prior to placing these in formalin.

Supplies

- Latex or nitrile gloves.
- Glass microscope slides with a frosted edge.
- A pencil.
- Scalpel blade.
- Immersion oil.

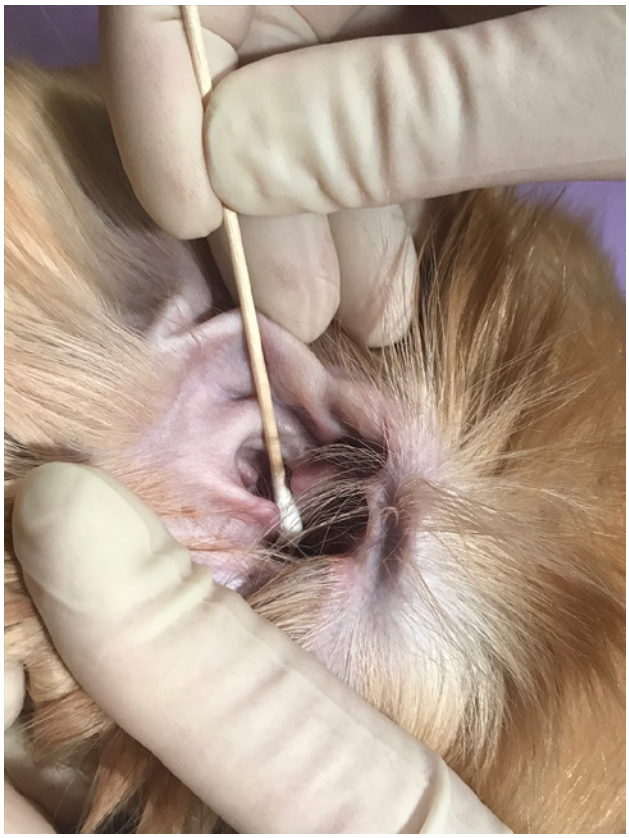


Figure 1.7 Swab collection method for external ear cytology.

Procedure Stabilize the skin or lesion with one hand and hold the scalpel in the dominant hand at a slight angle. Scrape the surface of the lesion with short, moderately firm strokes toward the angle of the tilted scalpel. For deep skin scrapings, continue to scrape until a small amount of serosanguinous material is noted, and collect this material with a scooping action using the scalpel blade.

Slide Preparation For superficial hair scrapings, place a small drop of immersion oil on the slide for the collected hair to remain in place. For deeper scrapings, use the scalpel blade to gently spread the material over the slide. If abundant material was collected and remains thick, use another glass slide to spread the material in a manner similar to that described in Section 1.2.3.1.

1.2.3.4 Impression Smears

These are often used to evaluate biopsy samples prior to being placed in formalin. They may be less useful for exuding lesions on the skin, as often only surface material is obtained, which may not be representative of deeper portions.

Supplies

- Latex or nitrile gloves.
- Glass microscope slides with a frosted edge.
- A pencil.
- ± Gauze swabs.

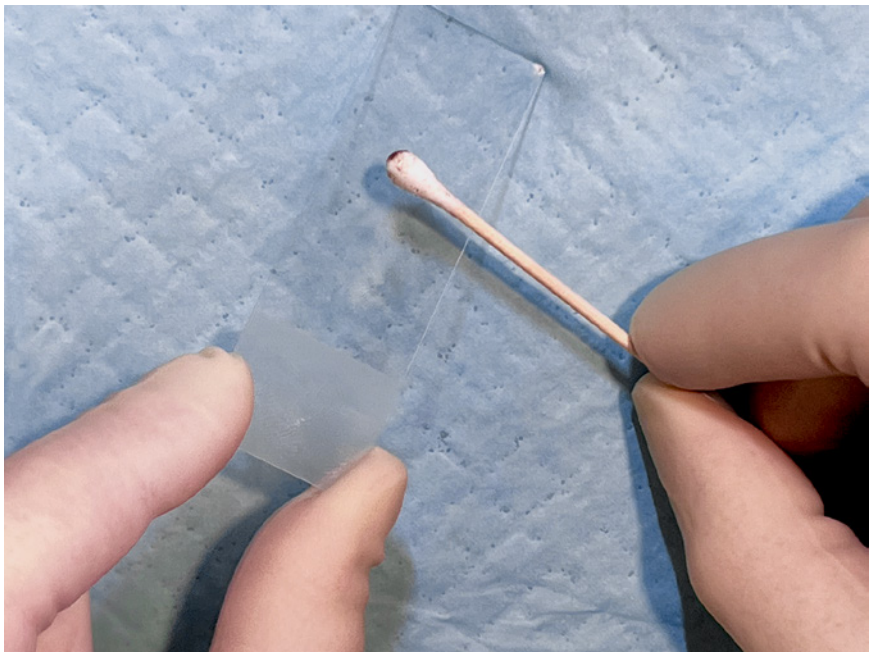


Figure 1.8 The swab is gently rolled along the slide to transfer material in a thin monolayer for review. Courtesy of Dr. Eric Franson.

Procedure and Slide Preparation For skin lesions, the slide is directly applied to the area, with care to avoid contamination from surrounding skin. Cleaning the lesions of any topical medications or excessive debris is recommended if possible. For biopsy samples, it is ideal to use a cut surface for access to cells deep within the tissue. Excess blood should be removed by blotting the cut surface on gauze prior to gently pressing on multiple areas of the glass slide.

1.2.3.5 Brushings

Cytology brushes are commercially available and can be useful for collecting cells from many surface locations, including ocular samples (cornea and conjunctiva) [16, 17] and the airways [18].

Supplies

- Latex or nitrile gloves.
- Glass microscope slides with a frosted edge.
- A pencil.
- Cytology brush.

Procedure The brush is gently moved and rotated over the surface of interest. Focusing on visibly abnormal areas free of excessive surface material (e.g., mucus or hemorrhage in the airways) will help maximize cellular yield.

Slide Preparation The material collected from the brushing procedure should be immediately transferred to the glass slides by gently rolling the brush in a thin linear row, similar to that described for swab samples in Section 1.2.3.2.

1.3 Sample Staining

Cytology samples are typically stained with Romanowsky-type stains for routine assessment. Examples of Romanowsky stains include Wright stain, Giemsa stain, May-Grünwald Giemsa, and Leishman stain. These stains contain eosin, which binds to basic components of the cells and stains them pink (e.g., hemoglobin in erythrocytes), and methylene blue, which binds to the acidic components of cells and stains them blue (e.g., nuclei).

1.3.1 Rapid Romanowsky-type Stains

Rapid Romanowsky-type stains are the most common stains used in clinic, for example, Diff-Quik (Siemens, Munich, Germany) and Hema 3® (Fisher Scientific, Pittsburgh, PA) (Figure 1.9). Methanol is used as fixative; however, these stains are aqueous-based.

1.3.1.1 Rapid Stain Procedure

The following steps detail how to maximize the stain quality of cytology samples using rapid Romanowsky-type stains:

1) Ensure slides are completely dry prior to staining.
2) Gloves should be worn when staining slides, and forceps or a wooden peg can be used to hold the slide during staining.
3) Fix slides for 1–2 minutes. Slides cannot be over-fixed; however, under-fixation of slides greatly affects stain quality and may lead to cellular lysis or poor stain uptake.

Figure 1.9 Rapid Romanowsky-type stains (Diff Quik).

Increasing fixation time greatly increases stain quality of the sample. Slides should be dipped in the fixative until evenly and completely covered and then allowed to sit in the fixative for 1–2 minutes. After this time, remove them and allow excess fixative to drain by standing the lower edge of the slide onto an absorbent pad.

4) Dip the slide into solution 1 (eosin) to completely and evenly cover it and then dip the slide a further 4–6 times for approximately 1 second per dip.
5) Allow excess stain to drain before repeating this step for solution 2 (methylene blue).
   Note: The number of dips and time spent in the eosin and methylene blue stains can and should be tailored to the needs of the sample and preference of the observer to increase or decrease stain intensity. For example, thicker preparations will likely require more time in each stain.
6) Rinse the slide thoroughly with water (ideally deionized water) and stand it vertically to dry.
   Note: Even if slides are being sent to a laboratory for evaluation, it is advisable to stain the slides one at a time and review them (until a diagnostic slide is confirmed) to ensure that intact cells are present and that the slide quality is adequate for interpretation, especially as non-diagnostic samples (often due to low or acellular samples) are frequently encountered in a diagnostic laboratory setting [19].

1.3.1.2 Advantages and Disadvantages

When using stains for cytology and hematology, it is important to appreciate their strengths and weaknesses, which can have an important impact on interpretation. The following lists summarize some of the important advantages and disadvantages of rapid Romanowsky-type stains [20].

Advantages

- Readily available and cost effective.
- Ease of use and fast turnaround time for staining samples.
- Good cytoplasmic detail.
- Excellent staining of background material and extracellular matrix.
- Typically stain infectious agents well (especially bacteria and Distemper viral inclusions) [21, 22].

Disadvantages

- Reduced nuclear detail (though nucleoli stain prominently).
- Diff-Quik may stain mast cell, basophil, and granular lymphocyte granules poorly, or not at all [21]. This is especially important when evaluating metastatic mast cell disease in lymph nodes [23].

1.3.2 Stain Care and Quality Assurance

Careful maintenance of stains dramatically affects stain quality. Ensure that fresh, clean stains are used to prevent excessive stain precipitation or contamination artifacts. Excessive stain precipitation (see Figure 2.9) may be seen on stained cytology slides with prolonged stain time, insufficient washing of slide, or use of old or unfiltered stains, and replacing or filtering stains on a regular basis is recommended.

Sometimes, changes in the color or intensity of the stain of blood smears may be appreciated: [24]

- Too pink: Low stain pH, inadequate time in methylene blue stain, degraded stain.
- Too blue: High stain pH, prolonged time in methylene blue stain, insufficient washing, exposure to formalin fumes.

1.4 Sample Handling and Storage

The following tips will help maintain slide quality during storage and transport:

- It is ideal to store and transport slides in plastic slide containers to prevent slide breakage or damage.
- If oil was used on the slides, resting the slides face down on microscope cleaning wipes for 1–2 hours will absorb much of the oil, allowing for cleaner storage.
- If slides are being kept for long-term use, store them in a dark, dry place. Dry, stained slides can be effectively and efficiently stored in empty microscope slide boxes with a reference list of the slides on a piece of paper that can be stored folded in the lid of the box.
- Never place slides in the refrigerator, especially if unfixed/unstained. Also avoid exposure to extreme temperatures, including leaving slides outside for transport pick up in winter or summer.
- It is imperative that unstained slides are not exposed to formalin fumes, which fix the cells and prevent appropriate uptake of cytologic stains, causing pale staining and preventing evaluation of nuclear and cytoplasmic detail (see Figure 2.19). Unstained cytology slides should therefore never be stored with histopathology samples in formalin and are ideally stored and transported in their own airtight bags.

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2

Cytologic Analysis of Cells

2.1 Approach to Cytology Samples

Adopting a routine approach to evaluating cytology samples makes cytopathology easier to approach, more efficient, and increases the chance of making a diagnosis. Four major components of every sample should be evaluated:

1) Sample quality and background.
2) Cell types.
3) Cell shape, distribution, and features.
4) Benign versus malignant.

2.2 Sample Quality and Background

The background of the sample is the first component to be evaluated, as it can provide important clues about underlying pathology. Some common background changes include:

- *Cystic material*: Cystic lesions often have a thick blue, purple, or pink background that may be scalloped. Cholesterol crystals are a hallmark of cell degeneration that occurs commonly in cystic lesions. These appear cytologically as rectangular, flat, non-staining crystals, often with a notched corner (Figure 2.1).
- *Necrosis*: Necrotic debris is seen as amorphous, globular, blue/purple/gray material (Figure 2.2). This material may predominate and obscure cellular detail.
- *Hemorrhage*: Blood often is present in the background of samples as a consequence of sampling, which may be supported if platelets are present. Prior hemorrhage within the lesion is confirmed when macrophages are erythrophagocytic and/or contain heme breakdown pigment such as hemosiderin or hematoidin (Figure 2.3).

Figure 2.1 Cholesterol crystals, 20× objective. Note the common notched corners.

Clinical Atlas of Small Animal Cytology and Hematology, Second Edition. Andrew G. Burton.

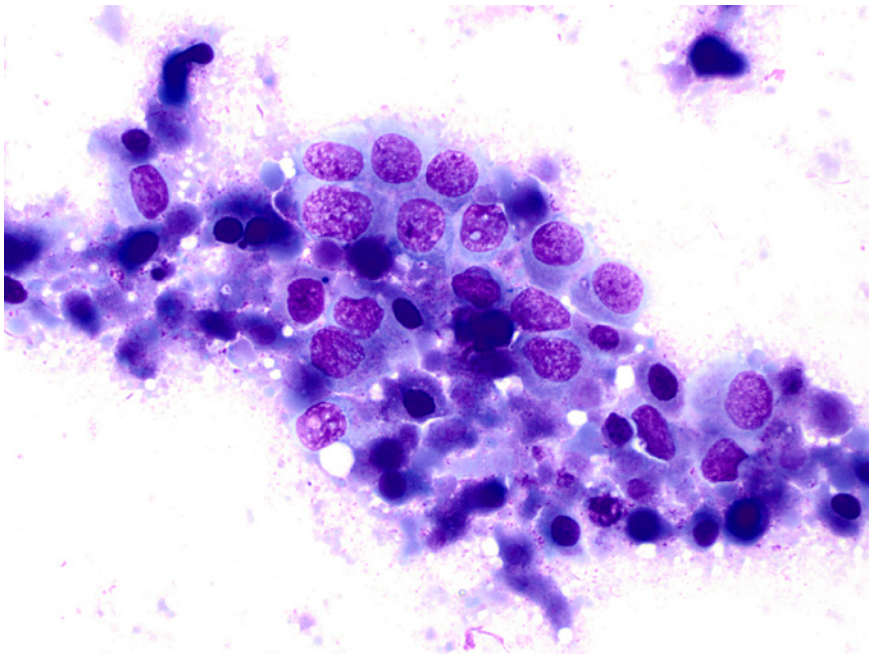

Figure 2.2 Necrotic material, 50× objective. Intact prostatic carcinoma cells are surrounded by globular, blue/purple necrotic material.

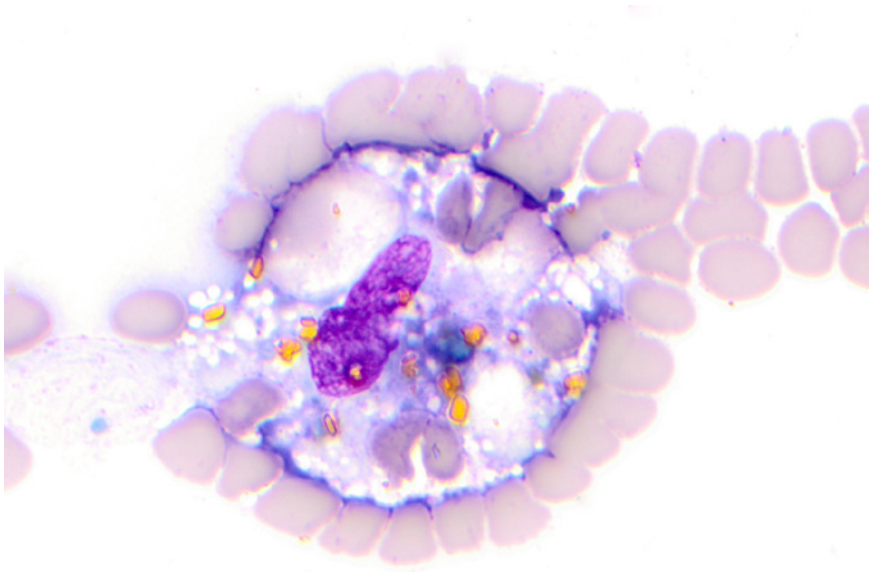

Figure 2.3 Hemorrhage, 100× objective. A macrophage is seen with a central purple nucleus. Red blood cells, blue/green hemosiderin, and golden hematoidin crystals are present within the cytoplasm.

- *Extracellular matrix*: Extracellular matrix is mostly bright pink and may be smooth, fibrillar, or stippled (Figure 2.4). It is most common with mesenchymal proliferation, but the basement membrane of epithelium can appear similarly. Collagen may also be seen, often as pale pink serpiginous ribbons (Figure 2.5).
- *Cytoplasmic fragments*: Fragments of cytoplasm (Figure 2.6) may be seen with any cell type; however, they are most commonly associated with lymphocytes (and have previously been called 'lymphoglandular bodies') [1].
- *Mineralization*: Mineralized debris is seen as clear, irregular/gritty, refractile aggregates, and often is seen outside the plane of focus of cells in the sample (Figure 2.7). It may be seen in chronic, cystic, or necrotic lesions.

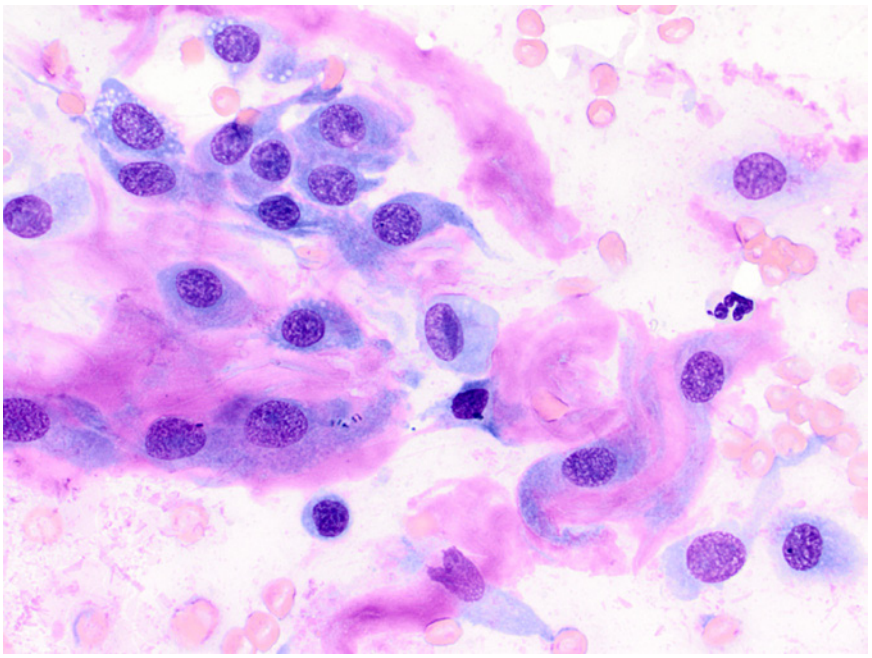

Figure 2.4 Extracellular matrix, 50× objective. Note the bright pink, smooth, streaming matrix material intimately associated with spindle cells (synovial cell sarcoma).

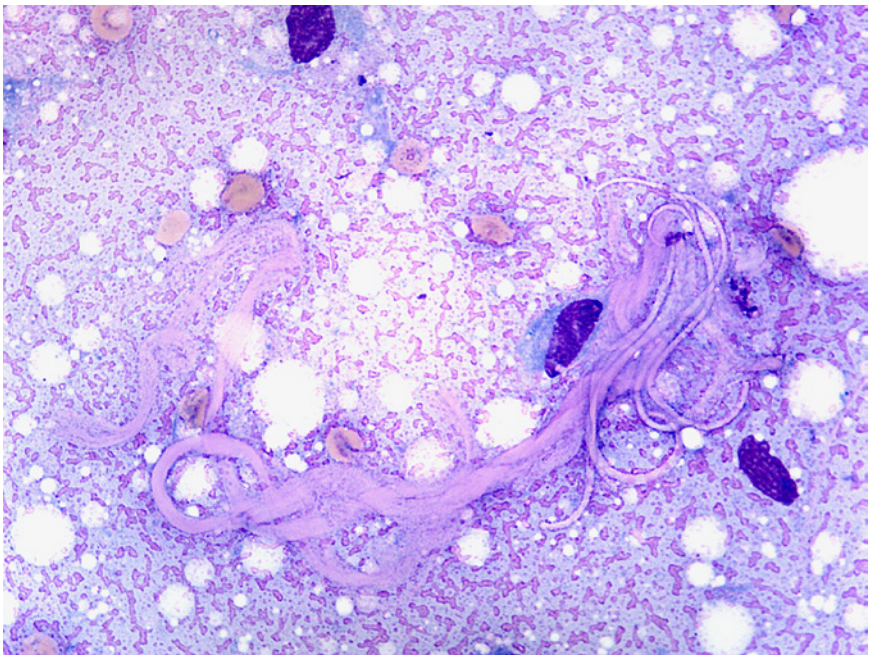

Figure 2.5 Collagen, 50× objective. Delicate tendrils of pink collagen swirl around well-differentiated mesenchymal cells (fibroma).

2.2.1 Artifacts and Incidental Findings

It is also important to recognize artifacts or incidental findings in samples. Glove powder starch crystals are round with a characteristic cross in the middle (Figure 2.8) and should be distinguished from mineralized debris. Precipitation of stain appears as variably coarse, granular pink/purple material (Figure 2.9). It may be seen in the background, but can cover cells, and care should be taken to distinguish it from cytoplasmic granules, intracellular bacteria, and lubricant/ultrasound gel which also appears granular/globular and stains bright purple/magenta (Figure 2.10). Crystals may form incidentally in the background of thick samples, making geometric patterns (Figure 2.11). Hemoglobin crystals may also form if

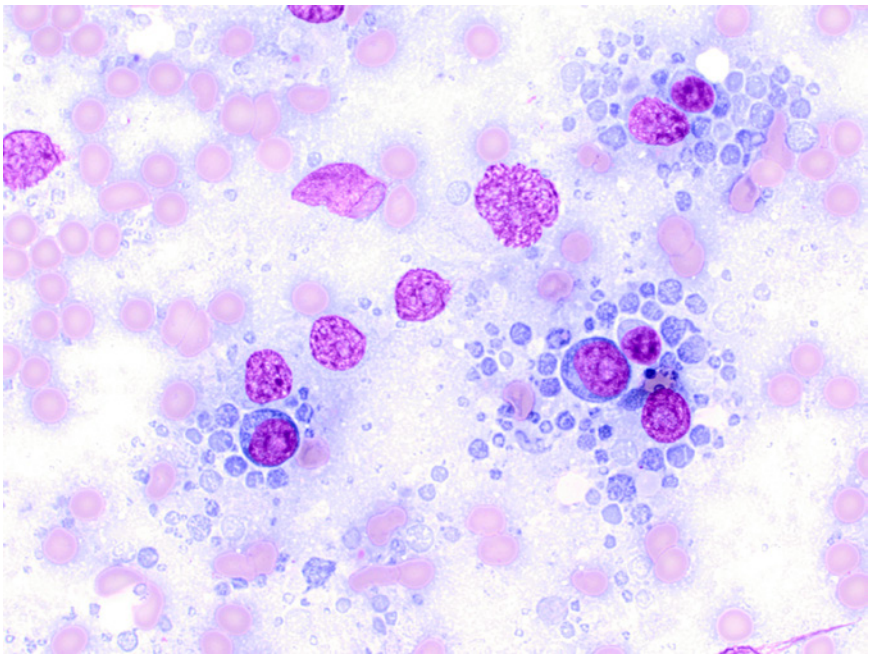

Figure 2.6 Cytoplasmic fragments, 50× objective. Note the small round fragments of pale-blue cytoplasm surrounding lymphocytes in a case of canine lymphoma.

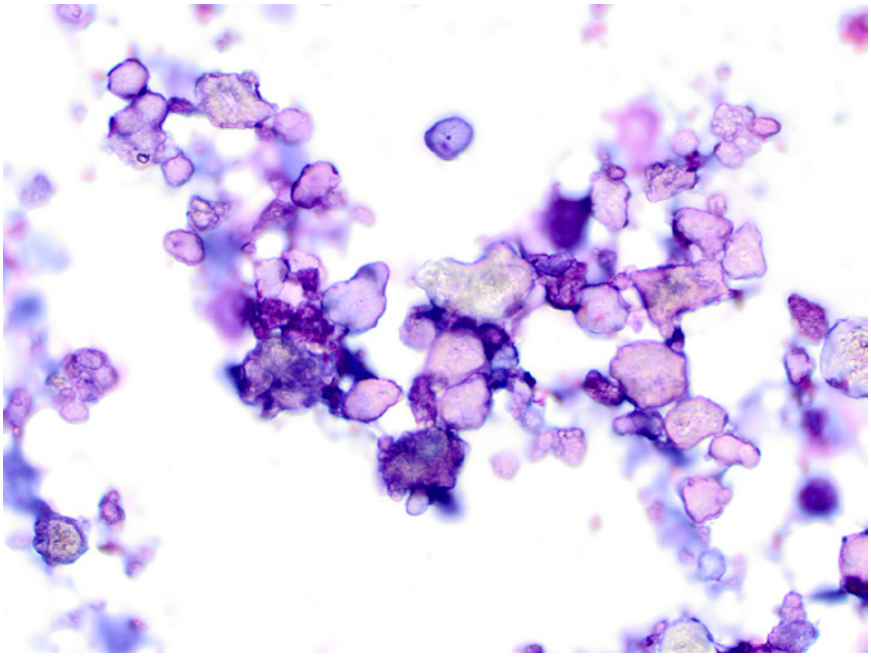

Figure 2.7 Mineralized debris, 50× objective. The material is seen in different planes of focus highlighting the three-dimensional nature of the mineralized debris.

hemodiluted samples are exposed to humidity or extreme temperatures (Figures 2.12 and 2.13), and clotted blood may produce striking colorful patterns (Figure 2.14). Other common artifacts/incidental findings include nuclear material and bare nuclei from lysed cells (Figures 2.15 and 2.16), apoptotic cells (Figure 2.17), and environmental contaminants including pollen grains (Figure 2.18). Formalin artifact results in a pale, homogeneous blue appearance to cells, and cellular detail is obscured (Figure 2.19). Cytologic samples should be transported separately from samples in formalin or should be in separate, airtight containers.