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Sample Collection and Preparation

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Evaluation of cytological samples has become well established as a method of obtaining a diagnosis of lesions in a wide variety of tissues. Cytology and histopathology will likely always remain complementary diagnostic procedures, reflecting a trade-off between the lower cost, reduced invasiveness of sample collection, and more rapid turnaround time with cytology and the increased amount of information available from the ability to evaluate tissue architecture with histopathology. However, the ever-increasing availability of advanced imaging techniques has resulted in an increased reliance on cytopathology to evaluate focal lesions of internal organs, which previously could not be reliably sampled. As clinicians have increased their use of this diagnostic modality and cytopathologists have become more experienced with the wider variety of lesions and tissues sampled, the spectrum of disease processes that can be identified by cytology and the reliability and precision of the diagnoses for lesions of many tissues have increased.

Other than the experience of the cytopathologist evaluating the samples, one of the major factors determining the diagnostic value of cytological specimens is the quality of the sample. The diagnostic yield of cytology is noticeably higher in the hands of clinicians who have a great deal of experience with obtaining cytological specimens. With histological specimens, once the tissue sample is collected and placed in an appropriate amount of formalin, laboratory technicians handle the remainder of sample preparation. With cytology, the clinician is faced with the responsibility of not only collecting an adequately representative specimen but also preparing the slides that are to be examined and, often, staining of the slides as well. Because the cells to be examined are not grossly visible during sample collection and slide preparation, it is often difficult to tell whether an adequate specimen has been obtained at the time of the sampling procedure.

Collection and preparation of cytological specimens is definitely a skill gained only through experience and refinement of technique based on the results obtained. Many clinicians (and owners) are understandably frustrated when a sample submitted is determined to be nondiagnostic. Fortunately, an understanding of some basic principles of sample collection and familiarity with some of the more common pitfalls related to cytological sample preparation can increase the odds of a diagnostic result.[1-5]

METHODS OF SAMPLE COLLECTION

Several methods of collecting samples for cytological analysis exist. The indications for each are outlined in Table 1.1.

Fine-Needle Biopsy

Fine-needle biopsy (FNB) can be performed by using a standard syringe and needle with or without aspiration (as described later). This is the best overall method for sampling any cutaneous mass or proliferative lesion.[1] FNB allows for collection of cells from deep within the lesion, avoiding surface contamination with inflammatory cells and organisms that often plague impression smears, swabs, or scrapings. Surface cells are often poorly preserved and may show artifacts related to cellular aging and exposure to secondary inflammation responses, especially with ulcerated masses. These changes can make evaluation of the significance of cellular atypia more difficult. A classic example of this is masses of the urinary bladder. Samples collected by traumatic catheterization often contain cells that show significant degeneration and artifact from aging and prolonged exposure to urine (Fig. 1.1). Conversely, samples collected via FNB from deep within the lesion are typically well preserved and easier to evaluate (see Fig. 1.1, A). FNB is also the only practical technique for sampling of subcutaneous or internal organs or masses.

Selection of Syringe and Needle

FNB specimens are collected with a 22- to 25-gauge needle and a 3- to 20-mL syringe. The softer the tissue, the smaller are the needle and syringe used. It is seldom necessary to use a needle larger than 22-gauge for aspiration, even for firm tissues. When needles larger than 22-gauge are used, tissue cores tend to be aspirated, resulting in a poor yield of free cells. Also, larger needles tend to cause greater blood contamination. For aspirating lesions deep within body cavities, longer needles may be needed, but the diameter should remain the same. Whenever aspirating lesions within body cavities, the needle may pass through, and collect cells from, nontarget organs. Serosal mesothelial cells are particularly common. Using a needle with a stylet in place and removing the stylet only when the lesion is entered can help reduce inadvertent collection of nontarget tissue.

The size of the syringe is not critical when the samples are collected by using the nonaspiration technique. If using the aspiration technique, the size of syringe used is influenced by the consistency of the tissue being aspirated. Softer tissues, such as lymph nodes, often can be aspirated with a 3-mL syringe. Firm tissues, such as fibromas and squamous cell carcinomas, require a larger syringe to maintain adequate negative pressure (suction) for collection of a sufficient number of cells. A 12-mL syringe is a good choice if the texture of the tissue is unknown.

Preparation of the Site for Aspiration

If microbiological tests are to be performed on a portion of the sample collected or a body cavity (peritoneal and thoracic cavities, joints, etc.) is to be penetrated, the area of aspiration is surgically prepped. Otherwise, skin preparation is essentially that required for vaccination or venipuncture. An alcohol swab can be used to clean the area. If the samples are being collected under ultrasound guidance, it is important to avoid the use of ultrasound gel, substituting alcohol as a

TABLE 1.1 Indications for Various Methods of Sample Collection

Collection Method	Indications for Uses	Comments
Fine-needle biopsy (aspiration or nonaspiration method)	Masses (surface or internal)	Best method for cutaneous or subcutaneous masses because it avoids surface contamination
	Lymph nodes	
	Internal organs	Best method for minimally invasive sampling of internal organs or masses
	Fluid collection	
Impression smear	Exudative cutaneous lesions	Most useful for identification of infectious organisms
		May yield only surface cells and contamination (problem with ulcerated tumors)
	Preparation of cytology samples from biopsy specimens	With biopsy specimens, it is imperative to blot excess blood from sample
		Impression smears of biopsy specimens must be made before exposure of biopsy sample to formalin
Scraping	Used with flat cutaneous lesions that are not amenable to fine-needle biopsy	With dry cutaneous lesions (e.g., ringworm), it is important to scrape sufficiently to obtain some blood or serum to help cells stick to slide
	Preparation of cytology samples from poorly exfoliative biopsy specimens	
Swab		Generally used only when anatomical location not amenable to collection by other means
	Vaginal smears	
	Fistulous tracts	With fistulous tracts, most useful in classifying type of inflammatory response and identifying infectious organisms

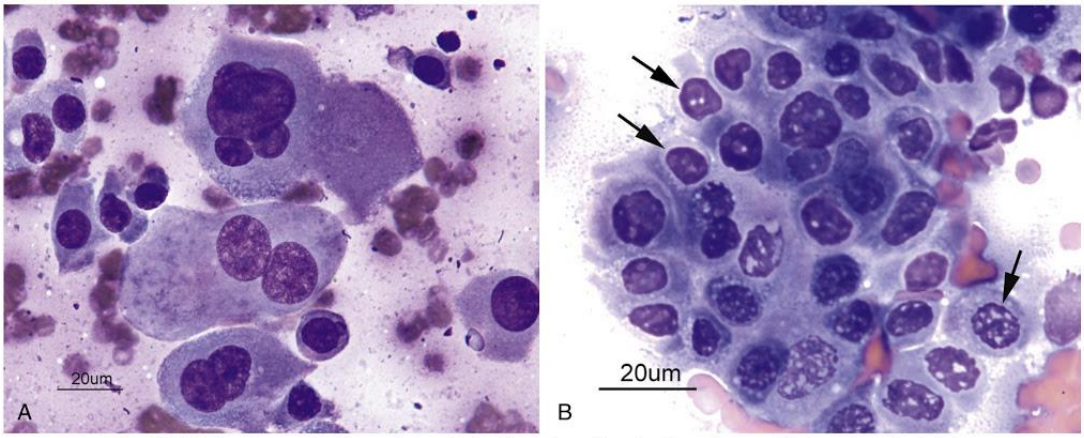

Fig. 1.1 Photomicrograph of samples collected from transitional cell carcinoma. (A) Sample collected by fine-needle biopsy of the mass. The cells are well preserved, allowing for examination of nuclear and cytoplasmic detail. (B) Sample collected by traumatic catheterization. These samples typically collect superficial cells that show marked changes resulting from cellular aging and exposure to urine. Nuclear degeneration is noted as a homogeneous light pink-purple color as well as fragmentation with numerous clear spaces evident *(arrows)*. (Courtesy Oklahoma State University teaching files.)

contact agent instead. Ultrasound gel stains pink with commonly used cytology stains. Even a small amount of ultrasound gel picked up as a contaminant when the needle passes through the skin is enough to completely obscure the cells and render a slide nondiagnostic.

Nonaspiration Procedure (Capillary Technique, Stab Technique)

Currently, most clinicians prefer to collect FNB specimens without the application of negative pressure. This technique yields samples of equal or better quality compared with those obtained with the older aspiration technique.[4-6] The nonaspiration technique works well for most masses, especially those that are highly vascular.[1] The procedure is performed by using a small-gauge needle on a 3- to 12-mL syringe. The barrel of the syringe is filled with air before the collection attempt to allow for rapid expulsion of material onto a glass slide. The syringe is grasped at or near the needle hub with the thumb and forefinger (much like holding a dart) to allow for maximal control (Fig. 1.2). The mass to be aspirated is stabilized with a free hand, and the needle is

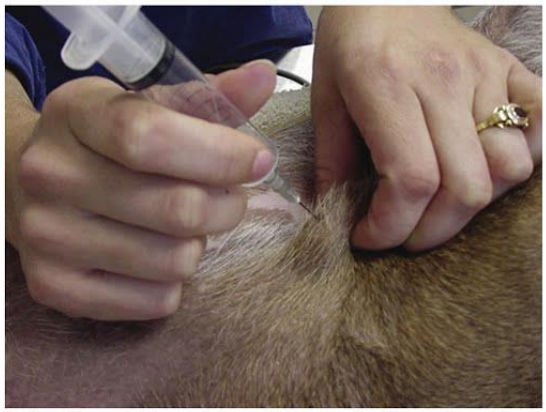

Fig. 1.2 Nonaspiration technique of fine-needle biopsy. The syringe is held at or near the needle hub with the thumb and forefinger. Note that the syringe is prefilled with air. The free hand is used to stabilize the mass. This technique allows greater control over movement of the needle. (Courtesy Oklahoma State University teaching files.)

Fig. 1.3 Aspiration technique of fine-needle biopsy. The mass is stabilized with one hand while the needle is introduced into the center of the mass. The hand holding the syringe is used to pull back on the plunger, creating negative pressure. (Courtesy Oklahoma State University teaching files.)

inserted into the mass. The needle is rapidly moved back and forth in a stabbing motion in an attempt to stay along the same tract, similar to the action of a sewing machine. This allows for collection of cells by cutting and tissue pressure. Care must be taken to keep the needle tip within the mass to prevent contamination with surrounding tissue. The needle is then withdrawn, the material in the needle is rapidly expelled onto a clean glass slide, and a smear is made by using one of the techniques listed later in this chapter (see "Preparation of Slides").

Having the syringe prefilled with air allows the sample to be expelled onto a slide more quickly, and this helps avoid desiccation (drying out) of the collected cells and coagulation of the sample.[6]

Some perform the nonaspiration technique with a needle only, with no syringe attached. This may allow for even greater control of the placement and movement of the needle, although the syringe must then be attached after sample collection to expel the material from the needle. Another variation that has been recommended for ultrasound-guided collection is to have an intravenous fluid extension set placed between the needle and the syringe.[6] This allows freedom of movement of the needle with one hand during the collection procedure. The syringe can be hung over the shoulder during collection, and then the other hand can be used to quickly expel the material onto the slide.

Aspiration Procedure

With the older aspiration method of FNB, the mass is stabilized with one hand while the needle, with syringe attached, is introduced into the center of the mass (Fig. 1.3). Strong negative pressure is applied by withdrawing the plunger to about three-fourths the volume of the syringe (Fig. 1.4). If the mass is sufficiently large and the patient sufficiently restrained, negative pressure can be maintained while the needle is moved back and forth repeatedly, passing through about two-thirds of the diameter of the mass. With large masses, the needle can be redirected to several areas within the mass to increase the amount of tissue sampled. Alternatively, several different areas of the mass can be sampled with separate collection attempts. Care should be taken to not allow the needle to exit the mass while negative pressure is being applied because this can result in either aspiration of the sample into the barrel of the syringe (where it may not be retrievable) or contamination of the sample with tissue surrounding the mass.

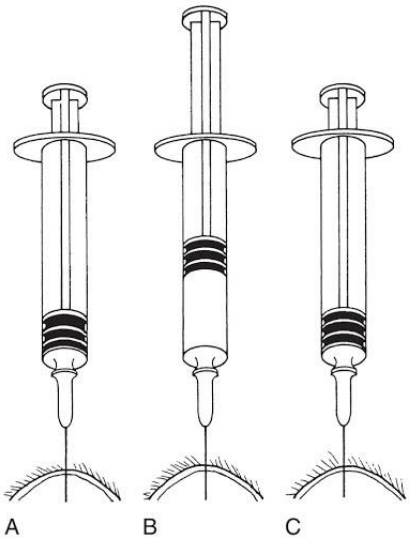

Fig. 1.4 Fine-needle aspiration from a solid mass. After the needle is within the mass (A), negative pressure is placed on the syringe by rapidly withdrawing the plunger (B), usually one-half to three-fourths the volume of the syringe barrel. The needle is redirected several times while negative pressure is maintained, if this can be accomplished without the needle's point leaving the mass. Before the needle is removed from the mass, the plunger is released, relieving negative pressure on the syringe (C).

The negative pressure should not be applied for more than a few seconds in any one area. Often, no material will be visible in the syringe or in the hub of the needle, even though an adequate sample has been obtained. With excessive force or prolonged application of negative pressure, disruption of blood vessels will eventually occur, and the sample will be contaminated with peripheral blood, diluting the tissue cells and rendering the sample nondiagnostic.

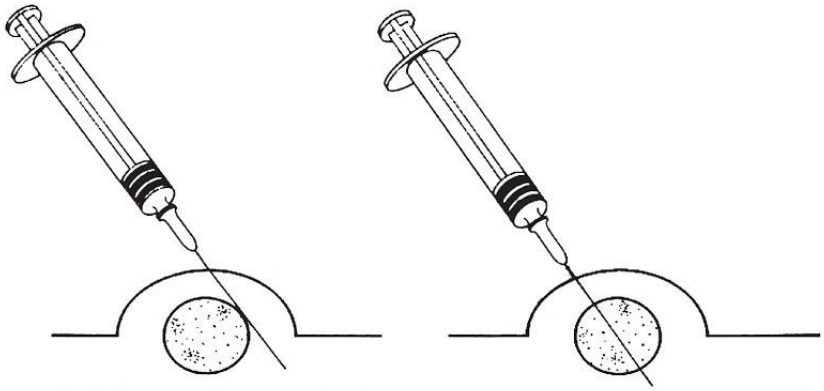

Fig. 1.5 Geographical miss. Sometimes, the needle is not in the area containing representative tissue of the lesion during sample collection. This is common in obese animals where the lesion may be surrounded by abundant subcutaneous fat. (Courtesy Oklahoma State University teaching files.)

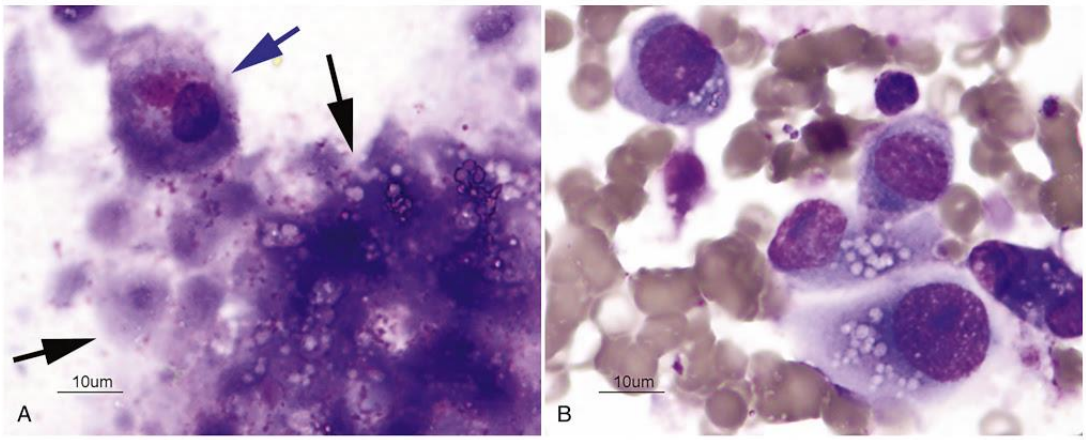

Fig. 1.6 Samples collected from a prostatic carcinoma with areas of necrosis. (A) Most slides were from aspirates of necrotic areas and contain predominantly necrotic cellular debris *(black arrows)*. A single partially intact cell is present *(blue arrow)*. These slides would be nondiagnostic. (B) One of the aspiration attempts sampled a nonnecrotic area, and the resulting slides contained numerous intact cells, allowing a diagnosis to be made. This demonstrates the importance of sampling multiple sites of a mass. (Courtesy Oklahoma State University teaching files.)

After several areas are sampled, the negative pressure is released, and the needle is removed from the mass and skin. The needle is removed from the syringe, and air is drawn into the syringe. The needle is replaced onto the syringe, and some of the tissue in the barrel and hub of the needle is expelled onto one end of a glass microscope slide by rapidly depressing the plunger. When possible, several preparations should be made, as described later in this chapter (see "Preparation of Slides").

If possible, it is optimal to perform multiple collection attempts at various sites within the mass to increase the chance of obtaining diagnostic material and to ensure a representative sampling of the lesion.

Collection Tips

Make and submit multiple slides. This is likely the single most important thing that can be done to increase the diagnostic yield. Small-gauge needles are used for collecting cytological specimens, and the procedure is usually relatively painless. It takes less time to perform several collection attempts and prepare multiple slides when the animal is first presented than to repeat a procedure after finding the specimen to be nondiagnostic, often after the animal has already been discharged from the hospital. This is particularly important if sedation or anesthesia is required for collection. It is optimal to stain and briefly examine one or two slides to ensure that they are adequately cellular while the patient is still in the hospital (or before animal has recovered, if anesthesia or sedation is required). If the slides stained are not cellular, additional collection attempts can be performed immediately.

There are many possible reasons for any one slide being nondiagnostic. The slide may not have any diagnostic cells because the needle missed the lesion during collection (geographical miss) (Fig. 1.5) or may have been in a nonrepresentative portion of the lesion (e.g., an area of inflammation or necrosis within a neoplasm (Fig. 1.6). In addition, some lesions simply do not exfoliate cells well. Even if adequate cells were collected, many times the cells do not spread out well and the slides are too thick to be evaluated (especially common in the case

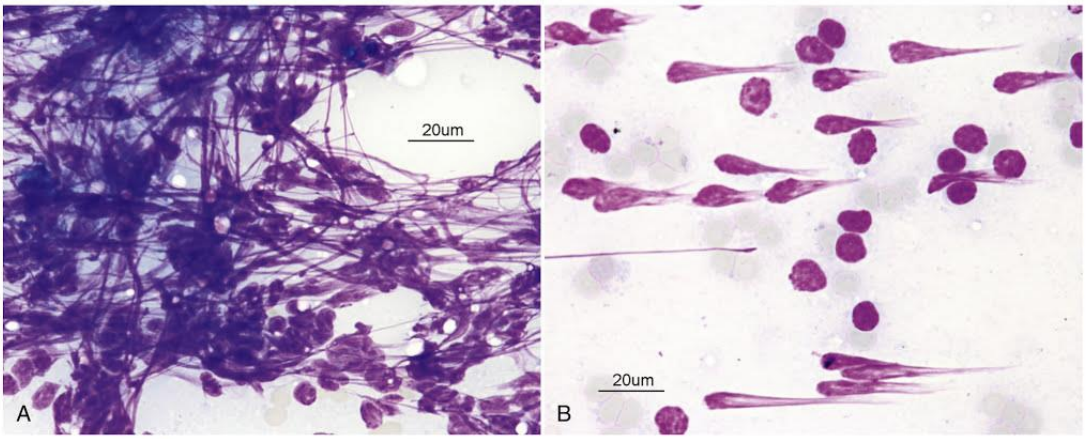

Fig. 1.7 Images from an aspirate of a reactive lymph node. This sample was nondiagnostic because all of the cells have been ruptured as a result of excessive downward pressure being applied during sample preparation. (A) The linear streaks of material represent nuclear chromatin of ruptured cells. (B) Ruptured cells often appear to have "comet tails" all going the same direction. (Courtesy Oklahoma State University teaching files.)

of lymph node aspirates), or all of the cells are ruptured during smear preparation (Fig. 1.7). Even in the hands of clinicians who are highly experienced in sample collection, it is not unusual to have multiple slides from a single lesion and all but one of the slides being nondiagnostic for one reason or another.

If possible, a minimum of four to five slides, representing collection attempts from several sites within the lesion, should be submitted from any lesion. If some of the samples appear to be excessively thick or if little to no material is apparent on the slides, additional slides should be made. With multiple slides, the chances of at least one of them being of diagnostic quality are increased.

If multiple masses are sampled, a new needle and syringe should be used with each mass. If this is not done, slides from one mass may be contaminated with cells left in the needle from previous collection attempts. Each slide should be clearly labeled as to the anatomical site sampled.

Avoid blood dilution. Blood contamination (hemodilution) is another common cause of nondiagnostic slides. FNB with aspiration will collect the tissue of least resistance. If blood vessels within the lesion have been ruptured, the tissue of least resistance will be peripheral blood. Once significant blood contamination has occurred, it is difficult to salvage the sample. Additional collection attempts should be made using a clean syringe and a clean needle.

The two major causes of blood contamination are the use of too large a needle (<22-gauge) and prolonged aspiration. Larger-bore needles do not usually collect more cells but are more likely to rupture small blood vessels. As mentioned before, material is often not visible in the syringe during sample collection despite adequate numbers of cells being present within the needle. Any time material is visible in the hub of the needle, the collection procedure should be stopped and slides made immediately.

Some lesions are highly vascular, making it difficult to avoid blood contamination, even with good collection technique. In these cases, use of a nonaspiration technique may result in less blood contamination and more tissue cells for evaluation.

Do not be timid. Other reasons for poor cellularity of a sample are inadequate negative pressure (aspiration technique) and slow or shallow needle passages (nonaspiration technique). When using the nonaspiration technique, the clinician is relying on the cutting action of the needle going through the tissue to create a slurry of cells and tissue fluid, which will enter the needle by capillary action. Needle passages should be quick and of sufficient length (although the size of the mass may limit the length of the needle pass).

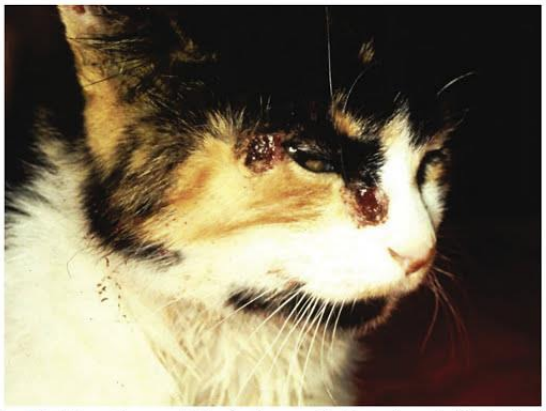

Fig. 1.8 Ulcerative, exudative lesions on the face of a cat. This lesion is well suited for impression smears. Slides from these lesions revealed inflammatory cells and many *Sporothrix* organisms. (Courtesy Oklahoma State University teaching files.)

Impression Smears

Impression smears can be made from ulcerated or exudative superficial lesions (Fig. 1.8) or tissue samples collected at surgery or necropsy (Fig. 1.9). Impression smears from superficial lesions often yield only inflammatory cells, even if the inflammation is a secondary process; neoplastic cells may not exfoliate in exudates or impression smears of ulcerated masses. If possible, FNB of tissue under the ulcerated or exudative area should be collected in addition to the impression smears. Inserting the needle at a nonulcerated area will help reduce contamination during collection. Impression smears of exudates or ulcers are

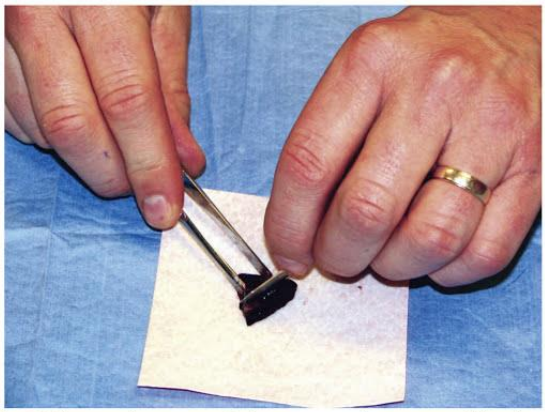

Fig. 1.9 Impression smear of tissue removed at surgery. The tissue is trimmed so that a fresh surface is created for making the impression smear. If normal tissue surrounding a mass has been excised, it is important to be sure that the tissue is cut through the area of interest. (Courtesy Oklahoma State University teaching files.)

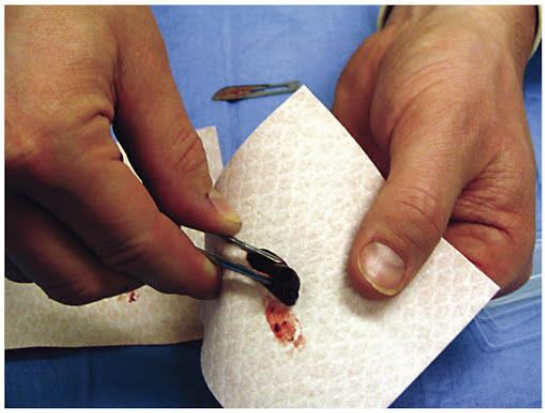

Fig. 1.10 The surface of the tissue is blotted several times against an absorbent material to remove excess blood and tissue fluid. This is extremely important to avoid slides that contain only peripheral blood. (Courtesy Oklahoma State University teaching files.)

most beneficial for determining whether bacterial or fungal organisms are present. Keep in mind that bacteria may reflect only a secondary bacterial infection.

Ulcerated areas should be imprinted before they are cleaned. The lesion should then be cleaned with a saline-moistened surgical sponge and reimprinted or scraped.

To collect impression smears from tissues collected during surgery or necropsy, the tissue should first be cut so that a fresh surface for imprinting is created (see Fig. 1.9). Next, the excess blood and tissue fluid should be removed from the surface of the lesion being imprinted by blotting with a clean absorbent material (Fig. 1.10). Excessive blood and tissue fluids inhibit tissue cells from adhering to the glass slide, producing a poorly cellular preparation. Also, excessive fluid inhibits cells from spreading and assuming the size and shape they usually have in air-dried smears. After excess blood and tissue fluids have been blotted from the surface of the lesion, the surface of the lesion is touched (pressed) against the middle of a clean glass microscope slide and lifted

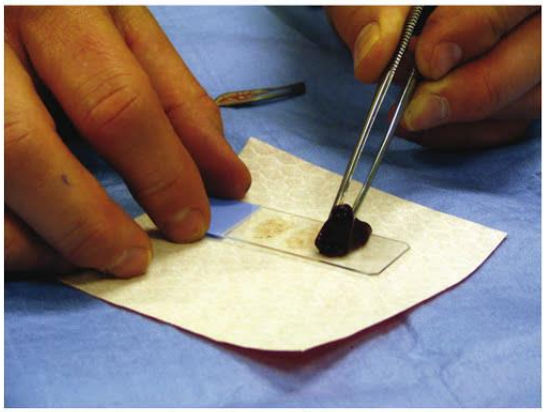

Fig. 1.11 The tissue is gently pressed (not smeared) several times against the surface of a clean glass slide. (Courtesy Oklahoma State University teaching files.)

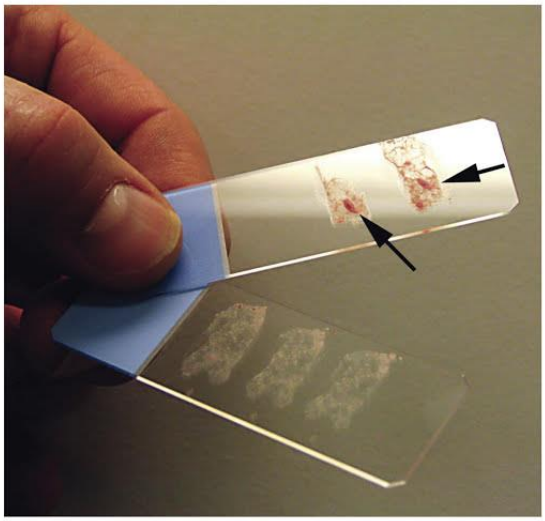

Fig. 1.12 The resulting slides from an impression smear. The slide on the bottom is properly made and has several slightly opaque areas where the tissue has been touched to the slide indicating cells have probably been transferred to the slide. The slide on top has excessive peripheral blood *(arrows)*, indicating that the tissue was not properly blotted against absorbent material before making the impression smear. This slide will likely contain only peripheral blood, or if cells are present, they may not be well spread out. (Courtesy Oklahoma State University teaching files.)

directly up (Fig. 1.11). This should be repeated several times so that several tissue imprints are present on the slide. If the excess blood has been adequately removed, the tissue will stick somewhat to the slide and will appear to peel off the slide, if removed slowly. Properly made slides will have slightly opaque areas at the areas of the impressions but should not have excessively thick areas of blood (Fig. 1.12).

No further smearing of the material is necessary. The tissue should not be allowed to slide around on the glass surface, as this causes cells to rupture. When possible, several slides should be imprinted so that a few can be retained in case special stains are necessary. After making

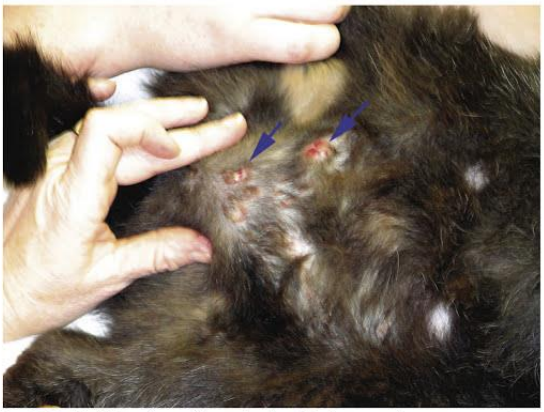

Fig. 1.13 Multiple plaquelike and raised lesions on the ventrum of a cat with eosinophilic granuloma lesions. The lesions were not thick enough to obtain good aspirates but yielded diagnostic cells via scraping. The ulcerated lesions are those that have already been scraped *(arrows)*. Scraping to the point of obtaining a small amount of blood or serum helps the cells adhere to the slides and also increases the chance of bypassing surface contamination and obtaining representative cells. (Courtesy Oklahoma State University teaching files.)

sufficient impression smears, the tissue used should be placed in an appropriate amount of formalin so that it may be submitted for histological evaluation, if necessary.

Scrapings

Scrapings can be made from external lesions or tissue obtained from surgery or necropsy. Generally, scrapings will result in more cellular slides than will impression smears; however, like impression smears, scrapings may contain mostly surface contamination or inflammation if made from the surface of ulcerated cutaneous lesions. Generally, scrapings are not as valuable for diagnosing neoplasia as slides made from FNB. Scrapings are valuable in collecting samples from cutaneous lesions that are flat and dry and thus not amenable to FNB or impression and from samples collected at surgery or necropsy (Fig. 1.13).[1] Two examples of lesions in which scrapings are beneficial are feline eosinophilic granuloma complex lesions and dermatophytosis.[7] Scrapings are prepared by holding a scalpel blade perpendicular to the lesion's surface and pulling the blade toward oneself several times. When scraping dry, nonulcerated lesions, such as dermatophyte lesions, scrapings should be sufficiently deep to cause exudation of serum or blood. This proteinaceous and fibrin-rich fluid will help the collected cells (and hairs when looking for dermatophytes) adhere to the slide and prevent them from being washed off during staining. The material collected on the blade is transferred to the middle of a glass microscope slide and spread either by smearing gently with the scalpel blade or by one of the techniques described later for preparation of smears from aspirates of solid masses.

Swabs

Generally, swabs are used only when other collection methods are not practical, as when obtaining samples from the vagina or the external ear or from within fistulous tracts. Swabs from the external ear canal and fistulous tracts are most useful for identifying infectious organisms. Specimens are collected from the site by using a sterile cotton swab. If the lesion is moist, the cotton swab need not be moistened. However, if the lesion is not very moist, moistening the swab with sterile saline is suggested because this helps minimize disruption of the cells that might occur during collection

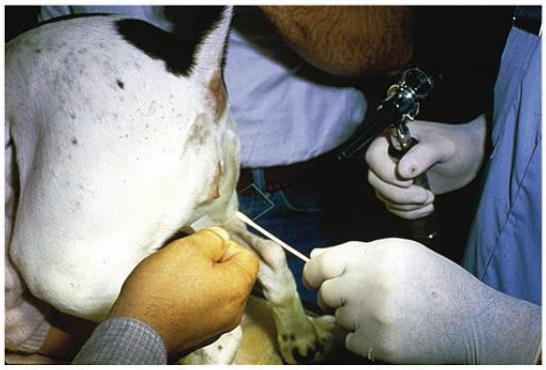

Fig. 1.14 Preparation of a vaginal swab from a dog. The swab containing the sample is gently rolled along the slide. Sliding or smearing the swab across the slide will result in excessive rupturing of the cells. (Courtesy Oklahoma State University teaching files.)

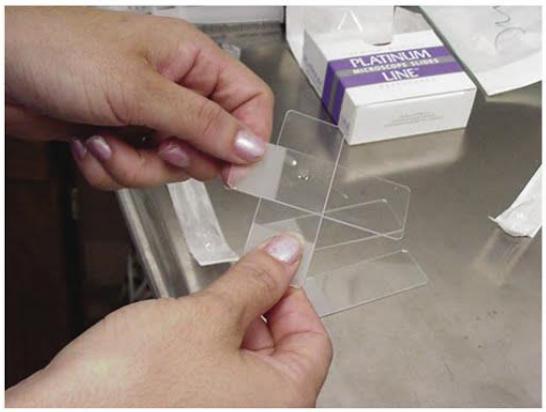

Fig. 1.15 Squash preparation. Once the sample has been placed on a clean glass slide, a second slide is placed on top of the sample and is used to spread out the sample. It is important that no downward pressure be applied on the top slide during spreading of the sample. (Courtesy Oklahoma State University teaching files.)

and sample preparation. Use of lubricant gels (e.g., K-Y Jelly) should be avoided when collecting swabs because they can coat the sample and interfere with staining of the cells, rendering the slide uninterpretable. Once the sample has been collected, the swab is gently rolled across the surface of a clean glass slide. It is important to not swipe the swab across the slide because this will often result in rupture of all the cells (Fig. 1.14).

PREPARATION OF SLIDES: SOLID TISSUE ASPIRATES

Slide-Over-Slide Smears ("Squash Preps")

When used properly, this is generally the best method for preparing slides from FNB or scrapings of solid tissue lesions. The goal is to prepare a thin film in which the cells are spread out into a single layer, without rupturing the cells. The material collected from the FNB procedure is expelled near one end (approximately one-half inch) of a clean glass slide (sample slide). A second glass slide (spreader slide) is placed on top of and perpendicular to the slide containing the sample directly over the specimen (Fig. 1.15). The specimen will usually spread

out between the two slides because of the weight of the spreader slide alone. If the sample is thick or granular and does not spread out well, light momentary downward pressure may be applied to the spreader slide and then released. The spreader slide is then lightly drawn out across the length of the bottom slide, spreading the sample (Fig. 1.16). Despite the (poorly worded) name "squash prep," it is important that no downward pressure be applied to the spreader slide while smearing the sample because this usually results in rupturing the majority of the cells.

If done properly, the smear should have a "flame shape" that does not extend to the edge of the slide. This is important because, as with a blood smear, often it is only at the edges of the sample where the cells are spread out sufficiently thin to be evaluated. Smears that extend off the edge of the slides are usually too thick to be evaluated. Also, many automated slide stainers do not stain the entire slide but leave an unstained area approximately one-quarter to one-half inch wide on either end of the slide. Cells in these areas will not be stained and therefore cannot be evaluated. Even when using dip-staining methods that stain the entire slide, material at the very edges of a slide may be impossible to view on some microscopes.

When done correctly, this technique does a good job of spreading out the cells, even those in clusters, so that cellular detail can be adequately evaluated. The main disadvantage of this method, particularly in inexperienced hands, is excessive cell rupturing. Lymphoid cells are particularly fragile and will often rupture if even moderate pressure is used when preparing slides with this technique.

Blood Smear Technique

With many samples, especially lymph node aspirates, the material expelled from the syringe onto the slide will have enough tissue fluid, blood, or both so that the sample can be smeared out as if making a blood smear (Fig. 1.17).[1] This technique will result in less cell rupturing, especially with fragile cell populations, and generally produces thin smears with intact cells that are well spread out.

As with the slide-over-slide technique, the sample is expelled from the syringe near one end of the sample slide. The long edge of the

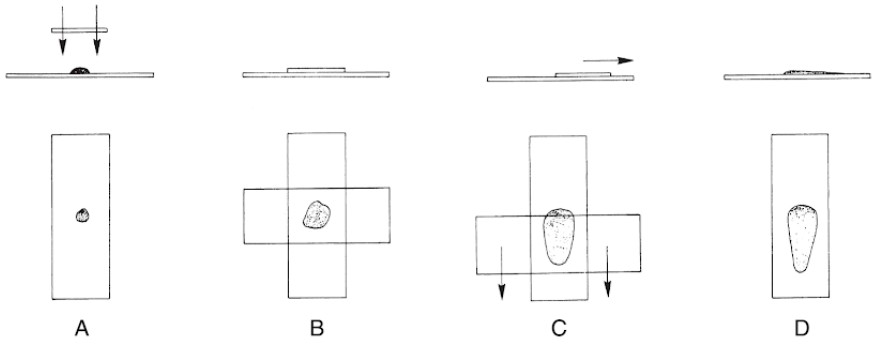

Fig. 1.16 Squash preparation. (A) A portion of the aspirate is expelled onto a glass microscope slide, and another slide is placed over the sample. (B) This spreads the sample. If the sample does not spread well, gentle digital pressure can be applied to the top slide. Care must be taken not to place excessive pressure on the slide, causing the cells to rupture. (C) The slides are smoothly slid apart. (D) This usually produces well-spread smears but may result in excessive cell rupture.

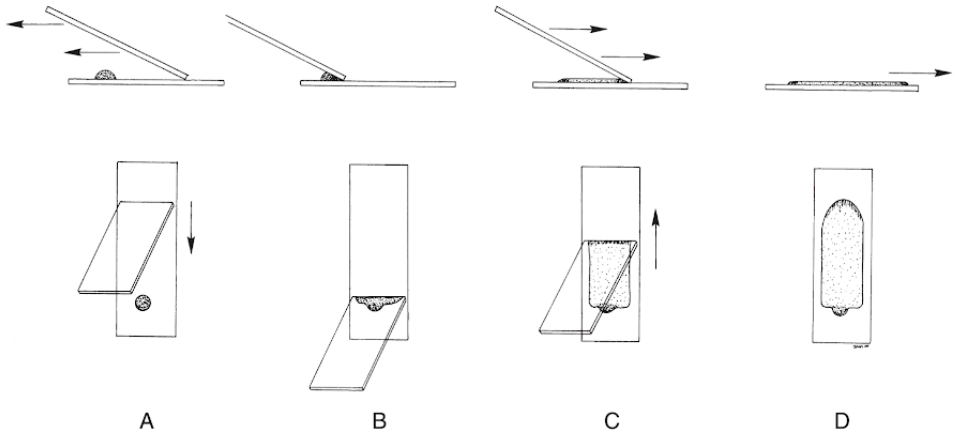

Fig. 1.17 Blood smear technique. (A) A drop of fluid sample is placed on a glass microscope slide close to one end, then another slide is slid backward to contact the front of the drop. (B) When the drop is contacted, it rapidly spreads along the juncture between the two slides. (C) and (D) The spreader slide is then smoothly and rapidly slid forward the length of the slide, producing a smear with a feathered edge.

spreader slide is placed onto the flat surface of the sample slide in front of the sample. The spreader slide is tilted to a 45-degree angle with respect to the sample slide and pulled backward about a third of the way into the aspirated material. The spreader slide is then smoothly and rapidly slid forward, as if making a blood smear. The smear should end in a feathered edge at least 0.5 inch from the opposite end of the spreader slide. If the sample smear extends all the way to the edge of a slide, additional slides should be made, and a smaller amount of sample should be put on the slide.

"Starfish" Preps

This is another technique used by some people, but generally is not the preferred technique because it may not produce good-quality smears. In this method, the aspirated material is dragged peripherally in several directions with the point of a syringe needle, producing a starfish shape (Figs. 1.18 and 1.19). This technique tends to avoid damaging fragile cells but allows a thick layer of tissue fluid to remain around the cells. Often, the thick layer of fluid prevents the cells from spreading well, and there may be few or no areas of the slides adequate for evaluation. Overall, a gentle slide-over-slide is recommended, but this technique can be considered if excessive cell rupturing is a problem.

Preparation Tips

Do Not Let the Sample Dry or Clot

If the sample clots or dries out on the slide before smears can be made, the cells may not spread out sufficiently to be evaluated. Also, the cells will often be distorted or not stain well because they are incorporated in a clot. Several clean slides should be laid out in an easily accessible area before the collection procedure to reduce the time between collections and final smear preparation.

One common mistake is to spray the sample from the needle onto the slide from a distance. This results in the sample being spread out in many small drops over the slide, much like a shotgun blast (Fig. 1.20). The problem is that these small drops tend to dry before the operator has time to make a smear. When viewed under the microscope, small clusters of cells appear poorly spread out (Fig. 1.21), and it is usually impossible to adequately visualize the morphology of the cells. When transferring the sample to the slide, the edge of the needle should be held very close to the slide, and the sample should be sprayed in one

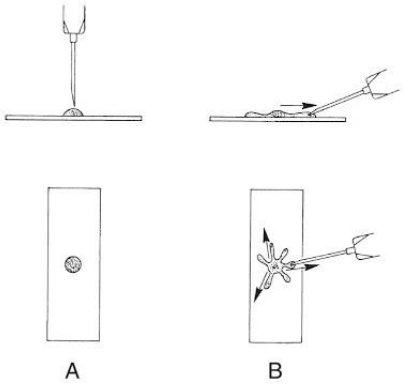

Fig. 1.18 Needle spread or "starfish" preparation. (A) A portion of the aspirate is expelled onto a glass microscope slide. (B) The tip of a needle is placed in the aspirate and moved peripherally, pulling a trail of the sample with it. This procedure is repeated in several directions, resulting in a preparation with multiple projections.

Fig. 1.19 Slide prepared using starfish or needle spread technique depicted in Fig. 1.18. Blue streaks indicating cellular areas are seen where the needle was dragged repeatedly across the slide and through the sample. (Courtesy Oklahoma State University teaching files.)

Fig. 1.20 Example of a poorly smeared sample. The slide on top is well made. However, the bottom slide shows what happens when a sample is sprayed onto the slide from a distance resulting in a shotgun blast–like arrangement of small drops. These drops dry quickly and then cannot be spread out. (Courtesy Oklahoma State University teaching files.)

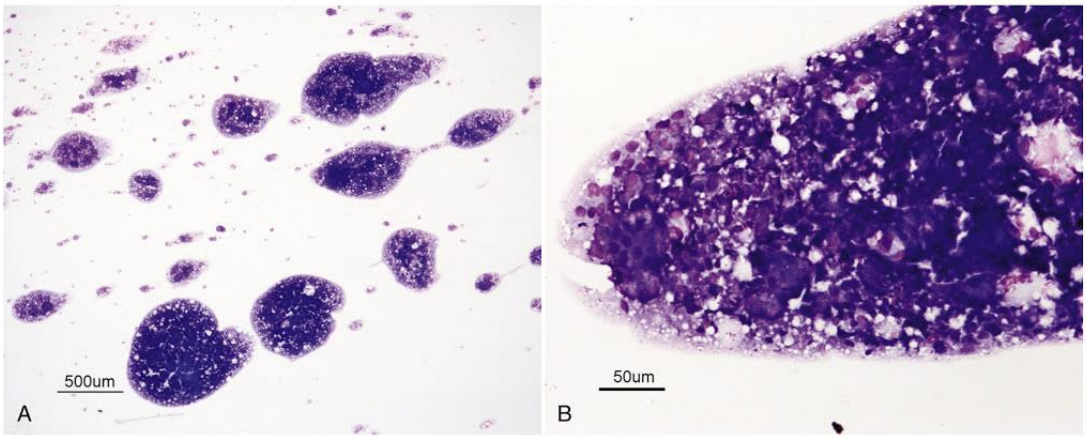

Fig. 1.21 Photomicrograph of slide shown on the bottom of Fig. 1.20. (A) Low-magnification image shows that the cells are all present in thick drops where the sample landed and that they were not spread out before the sample dried. (B) Higher-magnification image of one of the drops shows that the individual cells cannot be seen, resulting in a nondiagnostic sample. (Courtesy Oklahoma State University teaching files.)

drop, if possible. The sample should then be immediately smeared by using one of the techniques described previously. If sufficient sample is obtained to put on more than one slide, it is important to make all smears quickly before the sample dries. When using the nonaspiration technique, prefilling the syringe with air will shorten the time between sample collection and smear preparation and will reduce the likelihood of the sample clotting before smears can be made.

Avoid Making Too Thick a Smear

When smears are too thick, the cells will not spread out adequately, making it impossible to evaluate them. Samples that yield thick smears are those that are contaminated with excessive amounts of peripheral blood or samples collected from tissues that easily exfoliate large numbers of cells (e.g., lymph node aspirates). Ideally, only a small drop of sample should be applied to a slide (about the size of drop used in making a blood smear). If a large amount of sample is applied to a single smear, the smear generally ends up being too thick. If the sample extends all the way to the far end of the slide, the smear will probably be too thick.

Generally, the amount of sample being applied to the slide can be controlled when the material is expelled from the syringe. If too large a drop is applied to a slide, a thin smear can still be obtained by using the blood smear technique. The spreader slide is drawn back just to the point that it barely contacts the sample, which will begin to spread across the surface of the spreader slide by capillary action, and then is rapidly smeared forward. Alternatively, the spreader slide can be placed flat on top of the sample, as when preparing a slide-over-slide technique. Then, the spreader slide is lifted up and used to transfer a portion of the sample to another clean glass slide, on which a smear can be made. This technique can be repeated more than once, if needed, and finally the remaining material on the initial sample slide is smeared out. In this way, several thin smears can be made from one large drop of sample.

PREPARATION OF SLIDES: FLUID SAMPLES

A fluid sample can be obtained when sampling body cavities (e.g., thoracocentesis, joint tap), when performing washings (e.g., transtracheal wash), or when aspirating a cystic lesion (e.g., benign cyst, cystic tumor, sialocele). Proper handling of fluid samples is essential to obtaining diagnostic information. The two main considerations are preserving cell morphology during transit of the sample and preparing smears that are sufficiently cellular to allow for adequate evaluation.

Any fluid sample on which cytological evaluation is going to be performed should be placed in an appropriate amount of ethylenediaminetetraacetic acid (EDTA). EDTA will prevent coagulation of the sample (which can alter cell counts obtained from the specimen) and help preserve cell morphology during transport to the laboratory. This is especially important if the sample will be mailed. Usually, but not always, EDTA will adequately preserve cell morphology overnight and possibly longer. Refrigeration of the sample will extend the length of time that readable smears can be made from the sample. If culture of the fluid is anticipated, a portion of the sample should be placed separately into an appropriate transport medium or other sterile tube. It is important that a sufficient amount of sample fluid be added to the EDTA tube. EDTA has a very high refractive index, and if only a small amount of sample is added to a large EDTA tube, the total protein estimation determined by a refractometer will be artifactually elevated.

Even when fluid samples are placed in EDTA tubes and refrigerated or kept cool with ice packs, cells will undergo aging changes and eventually become too degenerate to be evaluated. Depending on the cellularity, type of cells present, and physical composition of the fluid (i.e., protein concentration), significant morphological changes may occur within 24 hours. The best way to preserve cell morphology is to send premade, air-dried smears. Once smears are made, cell morphology will be preserved for several days, even without fixation of the slides. If possible, premade smears should always be made and sent along with the fluid sample itself. Glass slides should never be placed in the refrigerator because condensation forming on the slide can result in lysis of the cells.

Fluid samples can vary from virtually acellular (cerebrospinal fluid) to extremely cellular (septic exudate). Depending on the nature of the sample, different techniques can be used to produce slides of adequate cellularity. Smears can be prepared directly from fresh, well-mixed fluid or from the sediment of a centrifuged sample using blood smear (direct smears) (see Fig. 1.17), line smear (Figs. 1.22 and 1.23), and squash prep (see Fig. 1.16) techniques. Table 1.2 outlines the samples to be prepared and submitted from fluid samples based on the characteristics of the specimen.

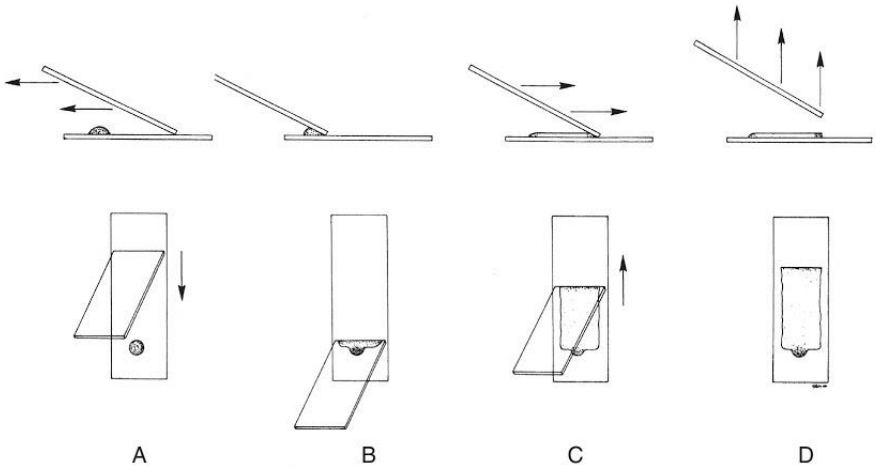

Fig. 1.22 Line smear concentration technique. (A) A drop of fluid sample is placed onto a glass microscope slide close to one end, and another slide is slid backward to contact the front of the drop. (B) When the drop is contacted, it rapidly spreads along the juncture between the two slides. (C) The spreader slide is then slid forward smoothly and rapidly. (D) After the spreader slide has been advanced about two-thirds to three-fourths of the distance required to make a smear with a feathered edge, the spreader slide is raised directly upward. This produces a smear with a line of concentrated cells at its end, instead of a feathered edge.

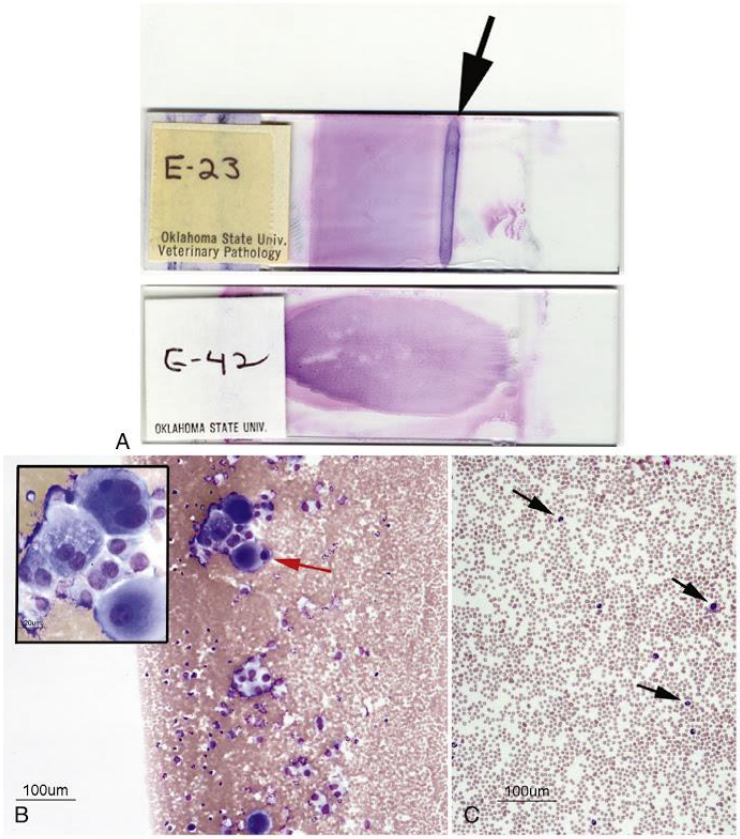

Fig. 1.23 Line smear made from fluid sample. (A) The slide on the bottom was made using a standard blood smear technique. Toward the right, the sample forms a typical feathered edge. The slide on the top was made using a line smear technique. Toward the right, the smear ends abruptly, forming a thick line with a higher concentration of large nucleated cells *(arrow)*. (B) and (C) Images taken from the line smear of a fluid sample. The main portion of the smear (C: right) is of low cellularity, consisting mostly of blood but with low numbers of nucleated cells *(black arrows)*. These relatively small cells are neutrophils and macrophages. At the line edge (B: left), there are increased numbers of nucleated cells, especially clusters of large neoplastic epithelial cells. Inset shows higher magnification of cell cluster indicated by the red arrow. (A, Courtesy Oklahoma State University teaching files.)

TABLE 1.2 Methods of Preparing Cytology Slides From Fluid Samples

Types or Characteristics of Fluid	Samples to Prepare or Submit
Peripheral blood for cytology	Make several air-dried direct smears (blood smear method).
	Submit remainder in EDTA.
Clear, transparent fluids (e.g., abdominal fluid)	Make one to two direct smears (can be used to estimate cellularity) and line smears.
	Centrifuge a portion of the sample and make smears from sediment.
	Submit a portion of fluid in EDTA.
	Submit a portion of fluid in sterile container if culture is desired.
Turbid or opaque fluids	Make one to two direct smears.
	Submit a portion of the fluid in EDTA.
	Submit a portion of the fluid in sterile container if culture is desired.
Clear fluid with flecks or mucous strands (e.g., transtracheal wash fluid, bone marrow samples in EDTA)	Make several direct smears from fluid.
	Make "squash preps" (slide-over-slide preps) of particles or mucous strands removed from the fluid using either a pipette, needle, or capillary tube.
	Submit a portion of the fluid in EDTA.
	Submit a portion of the fluid in sterile container if culture is desired.

EDTA, ethylenediaminetetraacetic acid.

Fig. 1.24 Direct smear *(left)* and concentrated smear made from sediment *(right)* of pleural effusion from a dog. The dark color on the concentrated smear is the result of markedly increased cellularity. (Courtesy Oklahoma State University teaching files.)

Blood Smear Technique (Direct Smears)

The blood smear technique (direct smear) usually produces well-spread smears of sufficient cellularity from homogeneous fluids containing 5000 cells per microliter (cells/μL) but often produces smears of insufficient cellularity from fluids containing less than 5000 cells/μL. The line smear technique can be used to concentrate fluids of low cellularity but often does not sufficiently spread cells from highly cellular fluids. In general, translucent fluids are of low to moderate cellularity, whereas opaque fluids are usually highly cellular. Therefore translucent fluids often require concentration, either by centrifugation or by the line smear technique. When possible, concentration by centrifugation is preferred. The "squash prep" technique often spreads viscous samples (e.g., transtracheal wash [TTW]) and samples with flecks of particulate material better compared with the blood smear and line smear techniques.

To prepare a smear by the blood smear technique, place a small drop of the fluid on a glass slide about 0.5 inch from the end. Slide another slide backward at a 30- to 40-degree angle until it contacts the drop. When the fluid flows sideways along the crease between the slides, slide the second slide forward quickly and smoothly until the fluid has all drained away from the second slide. This makes a smear with a feathered edge.

Sediment Preps (Centrifugation Preps)

To concentrate fluids by centrifugation, the fluid is centrifuged for 5 minutes at 165 to 360 × g (gravitational force). This is achieved by operating a centrifuge with a radial arm length of 14.6 centimeters (cm) (the arm length of most urine centrifuges) at 1000 to 1500 revolutions per minute (rpm). After centrifugation, the majority of the supernatant is separated from the sediment and analyzed for total protein concentration. The sediment is resuspended in a few drops of supernatant left in the tube by gently tapping the side of the tube. A drop of the resuspended sediment is placed on a slide, and a smear is made by the blood smear or squash prep technique (Fig. 1.24). Alternatively, a plastic pipette can be placed through the supernatant and used to remove the pellet of cells from the bottom of the tube and transfer it to a glass slide and make smears using a slide-over-slide technique. An absorbent tissue may be used to wick away excess supernatant before smear preparation, if needed.

Sediment-concentrated slides can produce highly cellular smears even from fluids of low cellularity and help identify cells present in low numbers (Fig. 1.25). When possible, several smears should be made by each technique. When slides are made from the sediment of a fluid sample, it is not possible to estimate the cellularity of the sample from the slides. Therefore it is imperative to either retain a portion of sample for cell counts or to make direct smears in addition to the sediment preps.

Line Smears

When the fluid cannot be concentrated by centrifugation or the centrifuged sample is of low cellularity, the line smear technique (see Fig. 1.22) can be used to concentrate cells in the smear. A drop of fluid is placed on a clean glass slide, and the blood smear technique is used, but the spreading slide is stopped and raised directly upward about three fourths of the way through the smear. This will result in a line containing a much higher concentration of cells than the rest of the slide (see