Wish you could interpret cytological specimens in practice rather than paying a lab to do it for you? Want to provide your clients with a faster service?

*Manual of Diagnostic Cytology of the Dog and Cat* is the ideal quick reference for the busy veterinarian in first opinion practice. It describes techniques for obtaining good quality cytological diagnostic specimens, and guides you through the interpretation of cytological findings.

Created to be used alongside the microscope, hundreds of high quality colour photos will help you to identify normal cell types and abnormal cytology, including both non-neoplastic and neoplastic lesions. It describes in a clear and concise manner the most common lesions and related disorders encountered in a practice setting. The concise format means that you can quickly find exactly what you are looking for.

Covering indications for cytological investigation, collection techniques and the evaluation and interpretation of findings, this concise manual will be your go-to resource.

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Manual of Diagnostic Cytology of the Dog and Cat
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Edited by

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During the last decade, diagnostic cytology has become an increasingly important and frequently used tool in the veterinary practitioner’s diagnostic armamentarium. *Manual of Diagnostic Cytology of the Dog and Cat* is aimed primarily at small animal clinicians and undergraduate veterinary students who wish to enhance their knowledge in this particular discipline, although clinical pathology residents should also find this manual useful.

The initial concept for this project evolved from the numerous requests received from veterinary clinicians in first opinion practice for a user-friendly and easily accessible reference source. The intention, therefore, has not been to compete with the numerous more comprehensive reference textbooks currently available on the market.

To this end, this manual reviews the techniques for obtaining diagnostic specimens and the general principles of cytological interpretation. It describes in a clear and concise manner the most common lesions and related disorders encountered in a practice setting. Considerable emphasis has been placed on the use of high-quality colour images. A conscious attempt has been made to describe the lesions in the figure legends rather than in the text thereby minimising the unnecessary repetition of facts. References are provided only where considered necessary. Readers are instead directed to the list of reference textbooks and articles for more in-depth information regarding each of the body systems.

Finally, I am indebted to the authors, all highly qualified experts in their field, for their excellent contributions. Thanks are also due to Nick Morgan and the editorial team at Wiley Blackwell, most notably Jessica Evans and Justinia Wood, for their forbearance in seeing this project through to its conclusion.

John Dunn
Because the final size of cells and other structures in digital photographs is variable in many cases due to cropping and resizing of photos, the magnification factors in the photographs throughout this manual are not given. The size of infectious agents and other cells can be compared to that of adjacent erythrocytes and leukocytes (see Figure 15.1).

The following stains have been used to stain the cytology specimens depicted in the following chapters unless stated otherwise in the figure legend:

Chapter 1: May–Grünwald–Giemsa stain
Chapter 2: Modified Wright’s stain
Chapter 3: May–Grünwald–Giemsa stain
Chapter 4: Wright’s–Giemsa stain
Chapter 5: Wright’s–Giemsa stain
Chapter 6: Wright’s–Giemsa stain
Chapter 7: Wright’s–Giemsa stain
Chapter 8: Wright’s–Giemsa stain
Chapter 9: Modified Wright’s stain
Chapter 10: Wright’s–Giemsa stain
Chapter 11: Modified Wright’s stain
Chapter 12: May–Grünwald–Giemsa stain
Chapter 13: Wright’s–Giemsa stain
Chapter 14: Pappenheim stain
Chapter 15: Wright’s stain
Acquisition of a fine-needle aspirate for cytological examination is a fast and easy, minimally invasive technique which can be performed in every practice or clinic. The advantages are that generally no anaesthesia or sedation is required and the risk of haemorrhage is minimal while the technique provides an excellent evaluation of single cell morphology. In contrast to histology, however, it has to be kept in mind that the tissue architecture is not preserved and cannot be evaluated. Histopathological examination of biopsy specimens allows the assessment of growth patterns and the margins of the lesion can be visualised if necessary, but surgical biopsy is associated with a higher risk of haemorrhage and anaesthesia (local or general) is necessary.

Adequate techniques of sample preparation and staining are mandatory for the optimal interpretation of cytological specimens. Moreover, correct interpretation of any cytological specimen requires correct microscopic examination and recognition of common artefacts. This chapter describes the practical approach to optimal sampling, routine staining techniques and the systematic microscopic evaluation and detection of common artefacts.

**Sampling techniques**

**Fine-needle aspiration**

Fine-needle aspiration cytology is a useful technique for the investigation of soft tissue masses (cutaneous lesions, lymph nodes, intra-thoracic or intra-abdominal masses) and effusions from body cavities. The technique can be easily performed in a practice setting. The following basic equipment is required:

- Glass slides with a frosted end which can be easily labelled.
- 5 ml syringe (if required also a 2 ml or 10 ml syringe; a 10 ml syringe might be advantageous for aspirating very firm masses),
20–22 G needles.

- A pencil for labelling the slides with the date and localisation of the lesion as well as the patient’s name. Note: Labels written with a ballpoint pen or marker may be washed away with alcohol-based stains (e.g. Diff-Quik, Wright’s, May–Grünwald–Giemsa).

For organs such as the liver or spleen, longer needles are usually required especially in large dogs. Here, a spinal needle with a stylet is recommended to avoid contamination by tissues adjacent to the mass or organ (with softer tissues smaller needles and syringes can be used).

Fine-needle aspirates can be taken with an ‘aspiration technique’ or a ‘non-aspiration technique’. The non-aspiration technique is preferred for sampling of all masses or organs which are highly vascular (e.g. spleen, liver) in order to minimise blood contamination. Overall, the sampling procedure should take no longer than 5–10 s, and several smears should be prepared.

- Aspiration technique:
  - The mass or organ (e.g. a peripheral lymph node) is immobilised with one hand and the needle is inserted with the other (Figure 1.1). Wherever possible, fine-needle aspiration of abdominal organs or masses is best performed under ultrasound guidance.
  - The skin is disinfected as for venipuncture.
  - The needle with attached syringe is inserted into the lesion.
  - The plunger is withdrawn, and while maintaining negative pressure, the needle can be redirected to aspirate different regions of the mass or organ.
  - The needle with attached syringe is removed after releasing the plunger.
  - The syringe is filled with approximately 3–5 ml air and reattached to the needle to expel the aspirate gently on the glass slide.

Figure 1.1 Fine-needle aspiration using a needle and syringe.
Note: To facilitate pulling the plunger, commercial aspiration guns may be useful when aspirating masses or organs which are difficult to immobilise since the vacuum can be easily maintained with one hand (Figure 1.2).

Non-aspiration technique: Two methods of this technique can be used for sampling.

‘Needle-alone technique’: The needle without the syringe attached is inserted into the lesion after disinfection of the skin (Figure 1.3). The needle is then rapidly moved back and forth in the tissue approximately ten times before it is withdrawn. A syringe already filled with 3–5 ml air ensures a rapid expulsion of the aspirated material onto the slide.

Figure 1.2 Fine-needle aspiration using an aspiration gun, e.g. ‘Zyto-Gun®’ (Scil animal care company GmbH, Viernheim, Germany).

Figure 1.3 The non-aspiration technique using a ‘needle-alone technique’ is useful for obtaining samples from small lesions such as pustules or bullae.
Alternatively, the needle is inserted with a syringe already filled with 2–3 ml of air attached (Figure 1.4). The needle and syringe are then rapidly moved back and forth in the tissue before the needle with syringe attached is removed. The aspirated material is then ejected onto the slide, and smears are prepared immediately.

Cytological smears can be prepared using the blood smear (Figure 1.5) or squash preparation technique (Figure 1.6).

**Impression smears/imprints**

Imprints can be made from wet surfaces (e.g. biopsies, ulcerated or exudative skin lesions) as well as from dry skin lesions using Sellotape (Figure 1.7 and Figure 1.8). It may be necessary to blot away excessive blood or tissue fluids from the surface of a biopsy specimen with a clean, dry swab or paper towel before making the imprint onto a clean glass slide. The disadvantages of impression smears are that they only collect cells from the surface of the lesion and therefore may not be representative of underlying pathology, fewer cells are collected and bacterial contamination is more likely.

**Scrapings**

Scrapings may be useful for sampling extremely firm lesions which are less likely to exfoliate cells with the aspiration technique. After the lesion is cleaned and dried, a large scalpel blade (held at a 90° angle) is moved several times over the surface of the
**Figure 1.5** Preparation of the smear using a blood smear technique. (A) The aspirated material is deposited onto the glass slide by ejecting 3–5 ml air through the syringe and needle. (B) A second slide held at a 45° angle (for highly viscous fluids such as joint fluid, smaller angles of approximately 25° are recommended) is brought towards until it makes contact with the aspirated material. (C/D) The material is distributed along the width of the spreader slide which is then pushed forwards smoothly and rapidly.

**Figure 1.6** The squash preparation technique. (A) The fine-needle aspirate is placed on the glass slide by ejecting 3–5 ml air through the syringe and needle. (B) A second slide is gently placed on top of the first one. Capillary forces result in the slides adhering to each other. (C) The top slide is gently drawn over the bottom slide on which the aspirate has been deposited. (D) The top (spreader) slide is removed once it reaches the end of the bottom slide which can then be submitted for cytological examination.
lesion in the direction of the person taking the sample. The scraped material is then transferred to a slide and is distributed evenly with the scalpel blade, or a second slide may be used to prepare a squash preparation using the technique described previously.

**Swab smears**

Swab smears are especially useful for preparing smears from fistulous tracts, the vagina or the ear canal (Figure 1.9). They are less useful for tumour diagnosis (the disadvantages are similar to those described for impression smears).
Brushings

Brushings are taken with a cytobrush (Figure 1.10) and have the advantage that they are more representative of the deeper layers of the lesion than lavage fluids, imprints or swabs. They are commonly taken from the conjunctiva, respiratory tract or vagina.

Collection and handling of fluid samples for cytological examination

The hair at the site of fine-needle aspiration is clipped and the skin is disinfected. If abdominocentesis is performed without ultrasonography, samples are taken on the linea alba 2 cm behind the umbilicus. Thoracocentesis without ultrasonographic guidance is performed at the ventral thoracic wall between the sixth and eighth ribs. The needle is inserted cranial to the rib to avoid injury to nerves or blood vessels. If larger amounts of fluid are aspirated, a three-way stopcock should be attached to the hub of the syringe to avoid creating a pneumothorax. Fluid specimens should be routinely collected into EDTA (or a plain tube if bacteriology is required). Smears should be prepared within 30 min of sampling to avoid artefacts due to sample aging.
For hypocellular fluids (e.g. bronchoalveolar lavage samples, cerebrospinal fluid), a cytospin preparation is preferred (Figure 1.11). For body cavity fluids (abdominal and thoracic effusions, synovial fluid), the cellularity can be estimated from a direct smear. If the cell count is low (i.e. < 10.0 × 10⁹/L), a cytospin preparation is recommended in addition to the direct smear to facilitate detection of cell populations present in low numbers. When, as will often be the case in a practice setting, a cytospin centrifuge is not available, a sample chamber for preparing an ‘in-house’ sediment smear can be prepared (Figure 1.12).

Although the results tend to be of lower quality than the in-house sedimentation or cytospin techniques, a sediment smear can also be prepared by centrifuging
the specimen for 5 min at 1000 g. After centrifugation, the supernatant is removed (decanted into the sink or aspirated with a pipette) so that a small amount (one to two drops) is left in the tube. The sediment is then resuspended in the residual supernatant, and smears are prepared using a blood smear or line concentration technique.

**Staining techniques**

Several stains are available which can be used alone or in combination. Prior to staining, the smears are air-dried; further fixation is generally not necessary.

Routinely used alcohol-based Romanowsky stains (Figure 1.13) include:

- May–Grünwald–Giemsa stain
- Wright’s stain
- Diff-Quik stain (Siemens Diagnostics Healthcare GmbH)

New methylene blue (NMB) stain (e.g. Accustain Reticulocyte Stain, Sigma Diagnostics, St. Louis) can be used to show up the nuclear and nucleolar structure (e.g. the nuclear chromatin) in greater detail. Typical staining characteristics of Diff-Quik stain, May–Grünwald–Giemsa stain and NMB stain are shown in Figure 1.14.
A simple staining technique using stains other than Diff-Quik (e.g. NMB stain) is as follows:

- The slide is placed on a tissue paper. The staining solution is added to the smear, e.g. with a micro-capillary.
- A cover slip is placed on top of the droplets of staining solution.
- Gentle pressure is applied to the cover slip, and the tissue paper is folded to absorb excess staining solution.
- The stained smear can be now evaluated under the microscope with a cover slip lens.

**Microscopic examination of cytological specimens**

Microscopic evaluation of the smears should always be performed in the same sequence:

After a macroscopic (‘eyeball’) evaluation of the smear to detect potential areas of interest (Figure 1.15A), the smear is scrutinised at low magnification with a
Figure 1.14  Staining characteristics of neutrophils (A) and hepatocytes (B) with Diff-Quik stain (1), May–Grünwald–Giemsa stain (2) and NMB stain (3). Diff-Quik and May–Grünwald–Giemsa stains show fairly similar results; however, chromatin structure of the nuclei and nucleoli can be seen in greater detail with the May–Grünwald–Giemsa stain. Moreover, mast cell granules do not always stain with Diff-Quik stain so that they may be misclassified as macrophages. Note that erythrocytes are clear in the NMB stain.

Figure 1.15  Microscopic examination of a smear (example is a splenic aspirate from a dog): (A) Step 1: Screen the smear macroscopically to detect areas with the most cellular material (circled). (B) Step 2: Screen the smear under low magnification using a 10× or 20× objective lens. In order to locate an area where cells are set in a monolayer and detect focal lesions (clusters of tumour cells, parasites such as microfilaria) Note: Large cellular populations tend to be found at the margins and at the feathered edge of the smear.
10× or 20× objective lens to detect areas in which cells are arranged in a monolayer and also areas which are hypercellular or have different staining characteristics (Figure 1.15B). These areas are then examined under higher magnification (with a 100× oil objective lens; Figure 1.16).

**Recognition of artefacts**

The recognition of artefacts is essential for correct interpretation of cytological findings. Artefacts may occur due to suboptimal sample preparation (e.g. crushing artefact results in smudged cells, ‘bare’ nuclei or cytoplasmic strands; Figure 1.17),
sample aging or contaminants such as stain precipitate (Figure 1.18), ultrasound gel (Figure 1.19), starch powder from surgical gloves (Figure 1.20), tissue paper or plant fibres (Figure 1.21) or pollen grains (Figure 1.22). Focusing and unfocusing will help to differentiate between contaminants and intracellular structures.

Figure 1.18  Precipitated staining solution (blood smear dog). Note the finely stippled azurophilic material on top and between the cells. Precipitated staining solution may be confused with *Haemoplasma* spp.; however, it can also be seen between the erythrocytes, and focusing up and down will confirm that the same material is on top of the erythrocytes.

Figure 1.19  Contamination of the smear with ultrasound gel (renal carcinoma, dog). Note the irregular lilac granular material in the centre of the photograph.
Figure 1.20 A starch powder grain (arrow) from surgical gloves is a contaminant in this fine-needle aspirate of a mass on the head of a grey parrot. Focusing and unfocusing reveals the typical 'cross' to y-shaped structure in the centre of the starch powder granule. Note: The starch powder grain is in focus, while the erythrocytes in the background are out of focus, indicating the contaminant is lying on top of the smear.

Figure 1.21 A plant fibre can be seen in this fine-needle aspirate of a renal carcinoma of a dog. This material might possibly be mistaken for fungal hyphae, but clear septation is missing, and focusing and unfocusing reveals that the material is placed on top of the cells.
The same applies for recognition of drying artefacts affecting erythrocytes which may appear as bluish intracytoplasmic structures resembling Haemoplasma organisms (Figure 1.23A); however, after unfocusing, they appear as refractile dots (Figure 1.23B).