

## 1

## Introduction to Dermatological Tests

Recent studies show that skin and ear diseases comprise 25% of all veterinary consultations. They are often complex and ongoing conditions that are a challenge to manage. Very few can be diagnosed on history and appearance alone. The modern approach to dermatology emphasises using the history and clinical signs to construct a logical differential diagnosis list. The diagnosis is then achieved by utilising appropriate tests to eliminate and/or confirm conditions in the differential diagnosis. On the other hand it is all too easy to become over-reliant on tests; it is most important that the clinical pathology is made to fit the history and clinical signs, not *vice versa*.

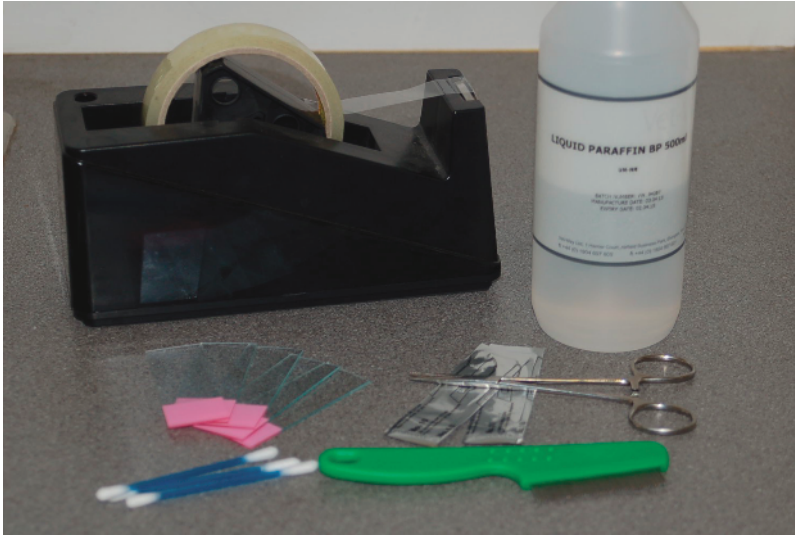
There is a very wide range of tests that can be used to investigate skin problems, but selecting, performing and interpreting the most appropriate tests in each case requires some experience. Many textbooks and journals, however, concentrate on individual skin conditions and assume that the reader is experienced enough to undertake the relevant diagnostic procedures. In practice, some of the most common reasons for poor management of skin conditions involve the inappropriate use of diagnostic tests, suboptimal execution of test procedures, inadequate sample choice and misinterpretation of results. The aim of this book, therefore, is to provide an illustrated, step-by-step guide to help you select, perform and interpret clinical tests and procedures for a range of dermatological presentations.

### What Equipment Will You Need?

A wide range of equipment is necessary for thorough examination of the skin, which may seem daunting. The vast majority, however, are inexpensive, non-specialist items that are common to virtually all veterinary practices and do not need special skills to operate. The few items that are expensive and/or need specific training to use are all optional; they are undoubtedly useful, especially to dermatology specialists, but are not necessary to successfully practise veterinary dermatology.

#### Essential Equipment

- Good lighting is essential for proper examination of the skin, lesions and collected material. Good fluorescent room lighting is a minimal requirement and a high-intensity spotlight is necessary for any serious examination.

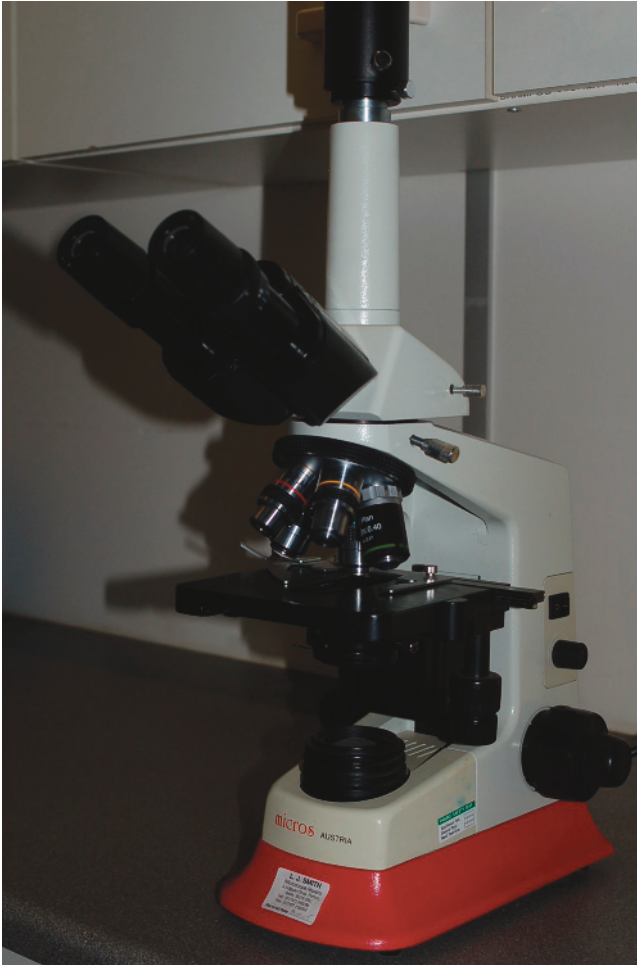


**Figure 1.1** Some equipment needed for taking samples for skin parasitology: a flea comb, clear sticky tape, No. 10 scapel blades, cotton buds, liquid paraffin, artery forceps for taking hair pluckings and microscopic slides.

- Flea comb for coat combings (Figure 1.1).
- Hand lens or magnifying glass for close examination of the skin, coat and collected material; the large illuminated lenses sold for reading are most useful (Figure 1.2).
- A good-quality binocular microscope for examining hair plucks, skin scrapes and cytology (Figure 1.3).

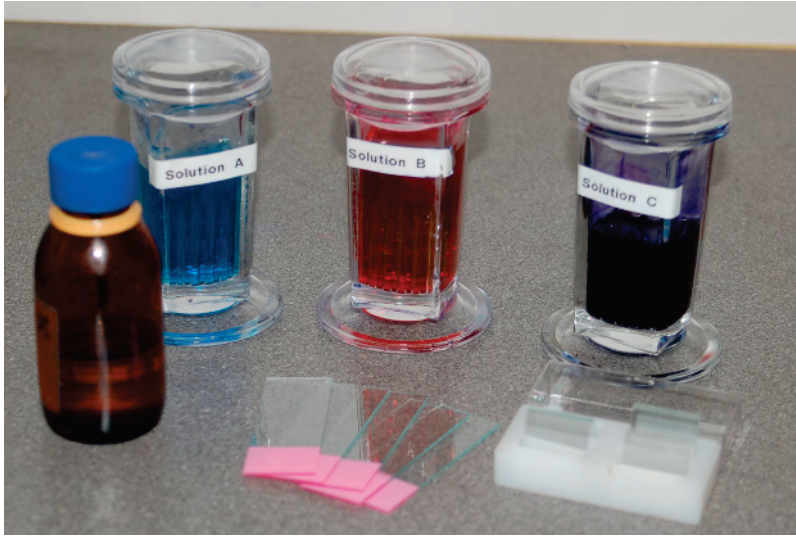


**Figure 1.2** A magnifying lens with illumination. In this case the lamp doubles up as a Wood's lamp for dermatophyte detection.



**Figure 1.3** A good-quality microscope is essential to be able to correctly identify parasites, cells and microorganisms in the samples examined. The image shows a trinocular microscope with the option to attach a camera to document the findings.

- Glass slides for mounting material; frosted slides are easier to label (Figure 1.1 and Figure 1.4).
- Cover-slips are essential for any microscopic examination (Figure 1.4).
- Immersion oil for using the  $\times 100$  oil immersion microscope lens; different types of oil with different viscosities are available. Type A is the least viscous and is often preferred as it is the least messy and the cheapest. Type NVH is the most viscous. For in-house use, type B would also be suitable also but it is messier than type A (Figure 1.4).
- Lens tissue or cloth and cleaning fluid or alcohol for cleaning microscope lenses without damage.
- Otoscope for examining the ears (Figure 1.5).
- Wood's lamp for screening for fluorescent dermatophytes (Figure 1.2).
- Electric clippers for removing hair, allowing access to the skin.



**Figure 1.4** Essential microscope equipment: glass slides with a frosted edge for easy labelling, glass cover-slips to improve the optic performance under the microscope, immersion oil and a modified Romanowsky-type rapid staining solution kit.



**Figure 1.5** Equipment used for otoscopy: a handheld otoscope, various sizes of cones to be attached to the otoscope (all packaged individually after autoclaving to sterilise the cones after each use) and a cone cleaner to remove otic debris after use prior to sterilising.

- Curved scissors for more precise and less traumatic hair removal.
- Fine-tipped curved artery (mosquito) forceps for hair plucks (Figure 1.1).
- Liquid paraffin for skin scrapes (Figure 1.1).
- 20% potassium hydroxide as a clearing agent when looking for parasites or dermatophytes (optional).

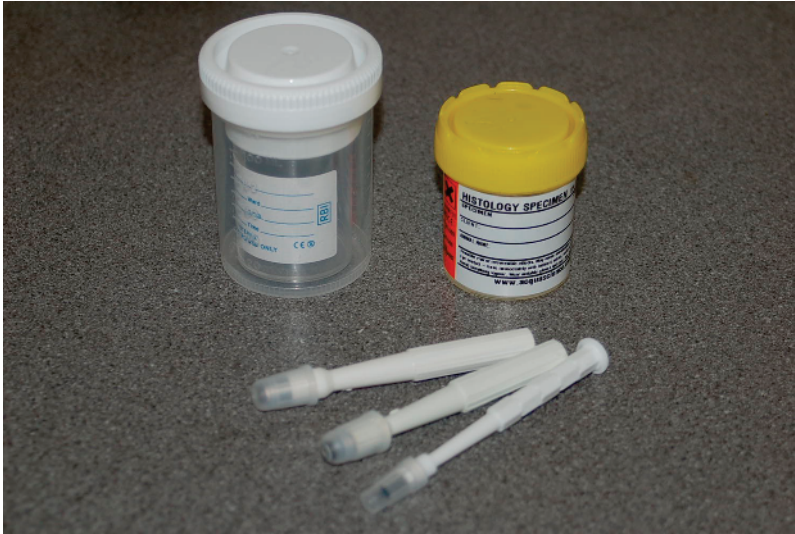


**Figure 1.6** Equipment for obtaining samples from the ear canal: cotton buds, bacteriology swabs with transport medium, liquid paraffin and glass slides.

- No. 10 and No. 15 scalpel blades for skin scrapes and biopsies (Figure 1.1).
- Cotton buds for collecting material from the ears (Figure 1.6).
- Adhesive tape (Sellotape®, Scotch Tape® etc.) for skin surface parasites and cytology (Figure 1.1).
- Sterile bacteriology swabs with and without transport media; fine-tipped ENT swabs are useful for taking samples from narrow sites or when using otoscopes etc. (Figure 1.6).
- Sterile universal (30 mL) and bijoux (5 mL) containers for storing and transporting tissue samples (Figure 1.7).
- Diff-Quik® type stain for routine cytology (Figure 1.4).
- Toothbrushes to collect material for dermatophyte culture (Figure 1.8).
- Syringes and needles of various sizes for taking blood samples and aspirates (Figure 1.9).
- Blood collection tubes: plain, EDTA, heparin and gel-clotting tubes.
- Indelible marker pen to mark biopsy and skin test sites.
- 4-, 6- and 8-mm skin biopsy punches (Figure 1.7).
- Basic surgical kit and suture material for performing skin biopsies and closing skin wounds.
- 10% neutral buffered formalin for fixing biopsy specimens (Figure 1.7).

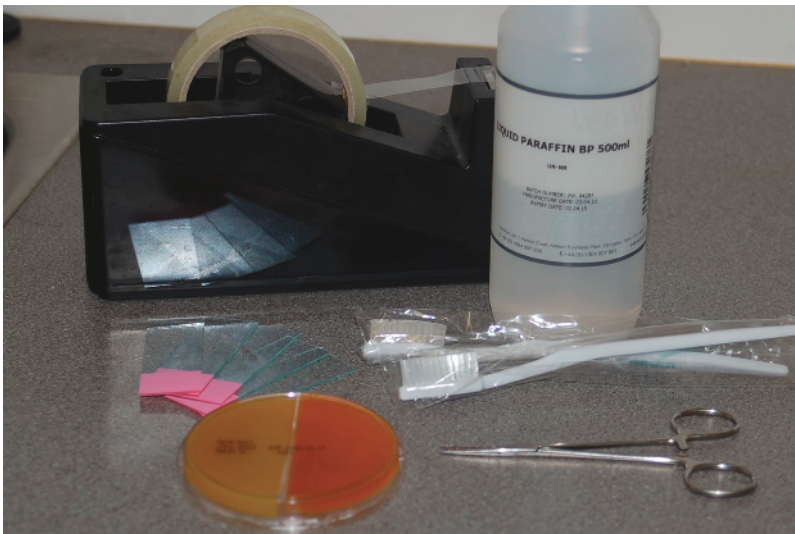
### Optional Equipment

- Dermatophyte test medium for in-house dermatophyte culture.
- Lactophenol cotton blue is useful for staining dermatophyte colonies for identification if in-house cultures are performed.



**Figure 1.7** Some equipment for obtaining and transporting tissue samples (skin biopsy specimens): single-use biopsy punches in varying sizes and sterile containers with formalin saline for histopathology or empty for tissue culture.

- Special laboratory stains for cytology: Grams, Leishman, Giemsa, Ziehl–Nielsen etc.
- Intradermal allergen test kit for more experienced clinicians with an interest in dermatology to perform allergy testing in atopic dermatitis (Figure 1.10).
- Video-otoscope (Figure 1.11).



**Figure 1.8** Materials to obtain samples for dermatophyte culture/perform in house cultures: clear sticky tape (for direct microscopy), liquid paraffin (for direct microscopy), sterile toothbrushes, artery forceps for hair plucks, glass slides for direct microscopy and a combined dermatophyte test medium (DTM) and Sabouraud agar plate for culture.



**Figure 1.9** Materials for fine-needle aspirates: different gauge sterile single-use needles, 5-ml syringes and glass slides.

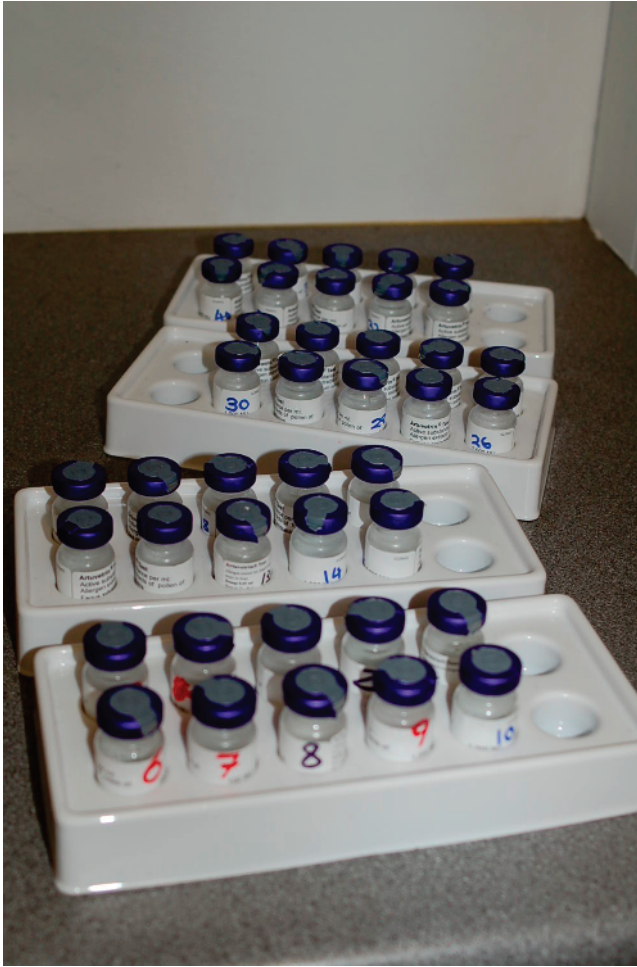
## Use and Abuse of the Practice Microscope

No piece of equipment is so vital or subject to so much abuse as a microscope. Robust and inexpensive models with binocular lenses and integral light sources are easily mastered and give good results, but need looking after and must be used correctly for the best results.

### Which Microscope Should I Buy?

Monocular microscopes or, even worse, those with mirrors for external lights, belong in a museum. The ideal microscope should have binocular eyepieces (with one capable of independent focusing), an integral light source, a focusing condenser, a mechanical stage, coarse and fine focus, and four lenses –  $\times 4$ ,  $\times 10$ ,  $\times 40$  and  $\times 100$  oil immersion (eyepieces are usually  $\times 10$  giving a final magnification of  $\times 40$  to  $\times 1000$ ). Dry (non-oil)  $\times 60$  lenses with a final magnification of  $\times 600$  are sometimes used instead of the oil immersion lens. Wide-field, stand-off eyepieces with rubber cups are best, as they can also be used when wearing glasses by folding the rubber cups down. The light source should provide white light (e.g. by using an LED) or have a daylight filter to convert the yellow–orange tungsten light to daylight (i.e. blue–white). More expensive microscopes have a filter mount above the light source so that a variety of other filters can be used, although this is rarely necessary in general practice.

You essentially get what you pay for with microscopes. Despite this, budget models with the above features are perfectly adequate for most routine practice use. Cheaper lenses, however, can result in a curved edge to the field of view, which can be disorientating and/or trigger motion sickness. More expensive, flat-field (or planform)



**Figure 1.10** Allergens for intradermal allergy testing. The relevant allergens will vary regionally.

lenses achieve an even depth of field across the whole image and avoid edge effects. More regular or intensive users may appreciate the increased robustness, finer movement and improved image that come with more expensive, better-quality models. It is often possible to try various models on loan first to select one that best fits your budget and requirements.

Trinocular mounts or multiple-headed microscopes are more expensive and are generally used where hands-on teaching or archiving of images is important. In general practice, however, cameras can also be useful for real-time video to show other staff and/or clients, and taking still images for clinical records or to send for a second opinion. The best results are from using trinocular mounts connected to digital cameras and monitors. It is also possible attach inexpensive CCD cameras to eyepieces and feed images directly to a computer, although the quality is not usually as good as using a dedicated camera and mount. Some camera-equipped mobile phones and tablets placed against an eyepiece can also take fairly decent images.



**Figure 1.11** A video-otoscope is very helpful for performing deep ear flushes.

### Microscope Set-Up: Where Should It Be?

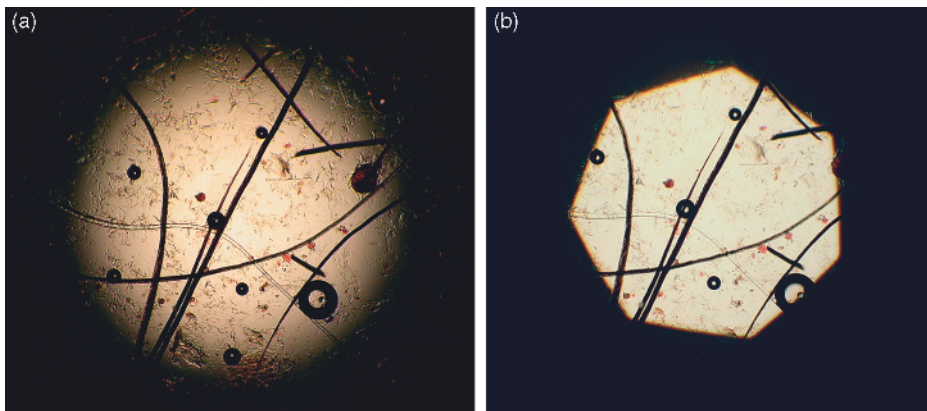
It is important that the microscope is situated where it is comfortable to use. Discomfort will lead to you skimming slides in order to finish them as quickly as possible or, in the worst case, not using the microscope at all. There should be a dedicated site for microscopy with enough working surface and shelving for the microscope, clinical notes, clinical slides and ancillary equipment, such as clean slides, cover slips, immersion oil and cleaning materials. The working surface should be firm and stable. An adjustable seat (ideally with lower back support) will allow different users to maintain a comfortable, upright position. Some users may like to use elbow or forearm pads; placing split foam tubing insulation over the edge of the bench can also help. It is important to examine and adjust the arrangements if using the microscope causes strain.

### Microscope Set-Up: Eye-Pieces and Illumination

It is also important to set the microscope up correctly. Incorrect adjustment can result in poor-quality images, eye strain, headaches and motion sickness. If these problems

cannot be solved by the fairly simple steps outlined below or cleaning, get the microscope professionally serviced.

- Alter the separation between the eyepieces until you see a single, circular image.
- Focus to get a sharp image with relaxed eyes; don't strain to get the image in focus.
- Adjust the focus between the eyepieces for your eyesight. First focus normally on a slide with one eye looking through the fixed (non-adjustable) eyepiece. Next, close your first eye and then correct the focus for your other eye, if necessary, by adjusting the other eyepiece (using its independent focus). You will now have the two binocular images in perfect focus for each eye. Some microscopes enable you to note down the adjustment, which is useful for multiple users with different eyesight.
- Koehler illumination (Figure 1.12) focuses the light source on the slide, giving the optical illusion that the light comes from the sample. This avoids transillumination and gives you the best light balance and image quality. It is very easy to set up:
  - close the light source lens diaphragm so that you can see the edges in the image field using the  $\times 4$  or  $\times 10$  objective;
  - focus the condenser until edges of the diaphragm are sharp;
  - adjust the condenser centring screws until the light source is centred in the field of view;
  - open the diaphragm so that it is no longer visible and there is even illumination of the field of view;
  - some older microscopes do not have a light source lens diaphragm – if this is the case, hold a thin piece of card, paper, a paper-clip, or the tip of a pen or pencil against the light source and focus the condenser until you see a sharp image outline in the image field.



**Figure 1.12** Koehler (Köhler) Illumination provides optimum contrast and image quality by focusing and spreading the light source evenly over the field of view. In these figures the light source diaphragm has been closed to set up Koehler illumination (in the absence of a light source diaphragm this can be approximated by holding a paperclip, piece of card or pencil point against the light source). a) The edge of the diaphragm is not in focus with the sample, which will result in poorer-quality images. b) Adjusting the condenser focus wheel (which moves the condenser lens independently of the slide stage) will bring the edge into focus and optimise the image.

- You can now adjust the iris diaphragm to give the clearest image for each lens:
  - for parasites and dermatophytes it is useful to close the iris diaphragm – the image is poorer but the increased contrast makes the parasites or fungi stand out better on unstained samples;
  - for cytology of stained preparations under high power, open the diaphragm to reduce contrast and improve the detail of the nucleus, cytoplasm, granules and microorganisms.

### Dark Field Microscopy

Dark field microscopy is rarely used in clinical practice and few practice microscopes have dark field capability. However, it can be useful in liquid phase non-stained preparations (e.g. urine sediments, *Leptospira*, *Treponema paraluis-cuniculi*, some endoparasites, insects in bedding, forage mites in foods etc.). It is also fairly easily to quickly modify most microscopes to do this.

The principle is that the centre of the light source is occluded, leaving the specimen illuminated only by scattered light from the margin. This gives a bright view of the specimen surrounded by a dark background. Dark field microscopes have occluding discs built in to the condenser. However, a similar effect can be achieved using discs of black card or insulation tape fixed to glass slides or discs and held against the light source, fitted to the light source filter holder or held against the base of the condenser. Ideally the edge of the disc should be just wider than the field of view for each lens. The condenser diaphragm and light intensity should be adjusted to give the best contrast for each view.

### Scanning the Field

You should always use a cover-slip (or cover glass). The only exceptions are tape-strip preparations where the tape is, in effect, its own cover-slip. Microscope lenses are designed to 'look' through a cover-slip and fluid layer, which provides a flat optical surface, puts material in a similar focal field, avoids air–fluid interfaces and reduces contrast. Curved surfaces (including oil on top of the cover-slip) act as mini-lenses and cause serious distortion. Cover-slips can also protect the lenses from scratches and the mounting fluid, and provide a defined search area for skin scrapes etc. Mounting fluids include immersion oil and liquid paraffin or potassium hydroxide, if these were used for skin scrapes or for clearing hairs prior to examination for dermatophytes etc. Cover-slips can be permanently sealed to slides using mounting solutions such as DPX (or, if necessary, clear nail varnish), but this is only really needed if the slides are to be archived. It is important to use enough mounting fluid to seal the cover-slip to the slide without gaps or air bubbles, but not so much that it gets onto the microscope lenses or stage. Be very careful not to get any DPX or similar mountant onto the microscope lenses or stage.

Initially, visually examine the slide to orientate it, and appreciate the depth of material, degree of staining and possible areas of interest. It is useful to check the quality of staining visually or under low power before placing a cover-slip, as the slide can be re-stained at this stage if necessary. You should then study the slide at progressively higher

magnification. Low-power lenses ( $\times 4$ – $\times 10$ ) are useful to scan large areas; with scrapes, plucks etc., start at one corner of the cover slip, proceeding down to the opposite edge, across one visual field and back up again to methodically search all the collected material. Use the high-power lens ( $\times 40$ ) to close in on areas of interest. Use the oil-immersion lens ( $\times 100$ ) last of all to avoid getting oil on the other lenses.

It is important that microscopic preparations are thin enough to allow proper examination without having to continually change focus. This is time consuming, tiring and diagnostic findings are easily missed. It is also time consuming to examine preparations that are too thin and spread out. It further follows that any material outside the area of the cover-slip will not be examined at all.

### Cleaning

Cleaning is really, really important. In the first instance, make every effort to avoid contaminating the rest of the microscope with oil and debris. Many non-oil immersion lenses are not oil-proof, and will need replacing if oil gets in. Dried oil and skin debris can also clog up and jam stages and focus wheels. Clean the lenses and stage with lens cleaner and lint-free, fine tissue or lens cloths immediately after use. Commercial lens cleaner is best, but methylated spirits or similar alcohols are also effective. Do not use anything that will leave a residue, or coarse paper or cloths that could damage the lenses or leave fine particles of lint etc. Dried on immersion oil and debris can be removed by carefully rubbing the lens on polystyrene soaked in lens cleaner.

## Stains

### Diff-Quik® Type Stains

There are several Diff-Quik® type stains available. These are modified Romanowsky or Wright–Giemsa stains used to identify cells and microorganisms. They are the most commonly used stains in practice by virtue of their ease of use and interpretation. There are three pots: fixer (usually methanol or methylated alcohol, which is pale blue to green), stain 1 (eosinophilic – red) and stain 2 (basophilic – deep blue to purple). Staining efficiency declines over time and the pots can also accumulate skin debris, *Malassezia* and bacteria that can contaminate slides. In a busy laboratory, dispose of old solutions, rinse the pots and replenish from the stock solutions every 1–2 weeks.

There is a variety of ways to dry, fix and stain cytology samples. These have their advantages and disadvantages, and to some extent the method depends on personal preference. However, it is helpful to think about the sample and the method that might be best in each situation.

### Two-Stain Method

Dip air-dried smears or tape-strips in each pot five to ten times for 1 second each time. The staining time will need to be longer for thicker, oily or waxy preparations. Rinse under a tap or with distilled water (directing the flow against the back of the slide stops

you flushing your preparation down the sink) and gently blot dry using textured paper towel without damaging the stained preparation. Wait for the rest of the water to evaporate or use a hand- or hair-dryer to completely dry the slide before applying a mounting agent and cover slip.

### One-Stain Method

With this method simply put a drop of the basophilic stain directly onto the cytology preparation, place a cover-slip and examine. Alternatively, the stain can be rinsed off and the preparation dried as above. With tape-strips, place one drop of the stain on the slide, stick the tape down over the stain, blot away the excess and examine. This approach is very quick and easy, and particularly suitable for tape-strips or waxy preparations that are hard to dry and stain. This method, however, leads to a more monochromatic stain. This is perfectly adequate for identifying microorganisms, such as bacteria and *Malassezia*, but distinguishing different cell types is harder.

### Checking Staining

Once the slide is ready, check the staining under low power; if it is adequate add a drop of oil and a cover slip; if not, re-stain. If necessary oil can be removed from slides for re-staining: place a piece of lens tissue on the slide, add one to two drops of xylene, acetone or methanol, allow the oil to pass into the lens tissue, and then carefully move it across the slide to remove the oil without damaging the smear. De-staining may be necessary if the slide is over-stained (the basophilic stain in particular can colour over cellular detail) or if using an alternative stain (e.g. Gram or Ziehl–Nielsen) is appropriate. Slides can be immersed in acetone, ethanol or methanol (you can also use the Diff-Quik<sup>®</sup> fixer in pot one) until sufficiently decolourised.

### Heat Fixing

Waxy or oily preparations can be difficult to air dry and, as they are soluble in alcohol, may be lost during fixing and using the two-stain method described earlier. Heat fixing, by passing the slide through a Bunsen or spirit burner or hand- or hair-dryer until it is just hand hot, and then staining with the eosinophilic and basophilic stains, can avoid this. Heat fixing can easily damage cytology preparations if it is overdone, however, and remember to let the slides cool before staining to avoid cracking them. Careful and thorough air drying using a hand- or hair-dryer and then staining in the usual fashion gives good results with most oily or waxy samples.

### Staining Tape-Strips

Tape-strips can be stained by directly immersing the tape in the solutions. This is most easily done by attaching the two ends of the tape to a microscope slide to form a loop on the surface or over the end of the slide. After rinsing, detach one end of the loop and stick the tape flat against the slide, blot out the excess water and examine. The tape traps a thin layer of water next to the slide, so it is not usually necessary to use a cover-slip. Most fixatives dissolve adhesive tape and are best avoided – if using the two-stain method (see earlier) just dip the tape in the eosinophilic and then the basophilic stain. The stains will also turn some tapes opaque, and it may be necessary to experiment to find a locally available, compatible combination.

### Other Romanowsky-Type Stains

Several other similar but distinct Romanowsky-type stains exist, but their use is mainly restricted to specialist laboratories as they are less straightforward and take longer than Diff-Quik® stains. **Wright's** (also known as Wright's–Giemsa) and **May–Grünwald** stains are particularly good at distinguishing fine cellular detail and are most frequently used for examining peripheral blood smears and bone marrow preparations. **Leishman's** (which is very similar to Wright's stain) and **Giemsa** are highly effective differential stains used to identify malaria, trypanosomes, other parasites and bacteria in peripheral blood smears, bone marrow specimens and other tissues. The stains are difficult and tedious to make up, and are best obtained from commercial sources.

### Gram Staining

Gram staining is a relatively simple technique, but with the advent of Diff-Quik® type stains its use in practice has become less common. The technique is widely used in bacteriology to differentiate Gram-negative (which stain pale red–pink) and Gram-positive (which stain purple–blue) bacteria. Cell wall damage, aging or death may cause Gram-positive bacteria to appear Gram-negative. Gram stains can be performed on smears prepared from tissue samples or cultured colonies. There are various methods, which vary in slight details. One technique is as follows.

- 1) Air dry and gently heat fix the smear by passing the slide briefly through a Bunsen flame until just hot to the touch.
- 2) Cover the slide with crystal violet solution for 1 minute. Briefly wash in tap water and drain.
- 3) Cover with Gram's iodine solution for 1 minute. Wash off with tap water, drain and then blot up remaining water.
- 4) To decolourise the preparation, cover with 95% alcohol for 10 seconds, wash off with tap water and drain. Thick smears may need longer, but be careful not to overdo it.
- 5) Cover the slide with safranin solution (the counterstain) for 30 seconds, wash off with tap water, and then drain and blot dry prior to microscope examination.

### Lactophenol Cotton Blue

Lactophenol cotton blue is widely used to stain fungal hyphae, microconidia and macroconidia for identification. It is commercially available and easy to use with preparations taken from fungal cultures in practice.

- 1) Place one to two drops of lactophenol cotton blue onto a microscope slide.
- 2) Tease a small amount of hyphae from the surface of a fungal culture into the stain.
- 3) Carefully place a cover-slip on the preparation for microscopic examination.

Clear adhesive tape is particularly good for collecting material for transfer to microscope slides and preserving the natural orientation of fungal elements.

- 1) Place one to two drops of lactophenol cotton blue onto a microscope slide.
- 2) Cut off a small piece of tape (approximately 0.5 cm<sup>2</sup>), taking care not to touch the adhesive surface.
- 3) Using forceps, gently touch the adhesive side to a fungal colony.

- 4) Lower the tape fungal (i.e. adhesive) side down onto the drops of stain and place a cover-slip for microscopic examination.
- 5) Alternatively, place the tape fungal side up, add one to two drops of stain onto the tape and then place the cover-slip.

### Ziehl–Nielsen Stain

Ziehl–Nielsen (ZN) staining is used to identify mycobacteria that stain poorly or not at all with Gram or Romanowsky stains because of the mycolic acid waxes in their cell wall. Heating allows the carbol fuchsin stain to penetrate the cell wall, where it forms a complex with the mycolic acids. This fails to decolourise with mild acid treatment – hence the term acid fast. ZN stains can be used on cytology smears, histopathology sections and culture material, but are rarely performed outside of histopathology or cytology laboratories.

- 1) Cover the sample preparation with carbol fuchsin stain.
- 2) Heat the stain so that it gently steams for at least 5 minutes. Add more stain if necessary to prevent the preparation drying out and do not let the sample boil.
- 3) Remove from the flame and allow to cool slightly to prevent the glass slide cracking in the next step.
- 4) Cover with acid–alcohol for 20 seconds then rinse in tap water to stop the decolourisation.
- 5) Counterstain with methylene blue for 60 seconds, then rinse in tap water and blot dry.

### Other Stains

There are various other stains that, again for reasons of time, expense and ease of use, are rarely performed outside of histopathology or cytology laboratories. Some stains that may be used include:

- **Haematoxylin and eosin (H&E)** is the most common tissue stain used in histopathology. Haematoxylin stains cell nuclei blue, while eosin stains cytoplasm, connective tissue and erythrocytes pink or red;
- **Periodic acid-Schiff (PAS)** stains carbohydrates such as glycogen, glycoprotein and proteoglycans. It is used to distinguish glycogen storage diseases and identify fungi in tissue sections;
- **Sudan stains** (such as Sudan III, Sudan IV, Oil Red O, and Sudan Black B) are used to detect lipids;
- **Methylene blue** is occasionally used to highlight nuclei in cytology specimens. It can also be used to stain hyphae, microconidia and macroconidia from cultured fungi.

Different staining methods are suitable for different types of samples and to examine the specimen for different types of cells and microorganisms. Table 1.1 summarises the uses of the various types of stain.

### Using Internal and External Laboratories

Using your in-house laboratory is quicker, but may not be any cheaper once the full running costs are taken into account, and may not be any easier or more accurate

**Table 1.1** Indications for the most common stains used on cytology samples and histopathology sections.

Stains	Uses
Modified Romanowsky or Wright-Giemsa (e.g. Diff-Quik <sup>®</sup> , Rapi-Diff <sup>®</sup> )	Routine rapid in-clinic staining of cytology samples and blood smears Differentiates cells, nucleus, cytoplasm, cytoplasmic granules, bacteria and fungi All organisms that stain with these stains will appear dark blue and the stains do not differentiate Gram-negative and Gram-positive bacteria
Haematoxylin and eosin (H&E)	Routine first-line histopathology stain May be combined with other stains to highlight specific organisms and substances
Gram	To identify bacteria in cytology samples and histopathology sections Differentiates Gram-negative and Gram-positive bacteria
Ziehl–Nielsen	To identify acid-fast bacteria, mainly mycobacteria but also <i>Nocardia</i> and <i>Rhodococcus</i> , and some protozoa (e.g. <i>Cryptosporidium</i> )
Grocott–Gomori’s methenamine silver	Stains both living and dead fungal organisms
Periodic acid–Schiff (PAS)	Detects polysaccharides (e.g. glycogen), glycoproteins, glycolipids and mucins in histopathology sections Will stain living but not dead fungi
Giemsa	Accurate identification and differentiation of cells in cytology samples, bone marrow aspirates and blood smears To identify certain microorganisms (e.g. trypanosomes, spirochaetes, <i>Histoplasma</i> , <i>Chlamydia</i> and <i>Chlamydophila</i> , and protozoa)
Wright	Accurate identification and differentiation of cells in blood smears Combination Wright–Giemsa staining is widely used
Leishman	To identify leucocytes, blood-borne parasites and trypanosomes in blood smears
Sudan (Sudan II, Sudan III, Sudan IV, Oil Red O, and Sudan Black B)	To identify fat in cytology samples, histopathology sections and faeces
Congo red	To detect amyloid in histopathology sections
Toluidine blue	Detects mast cell granules in cytology samples and histopathology sections
Calcofluor-white	Fluorescent stain that identifies fungi, algae and related organisms on hairs, cytology samples and histopathology sections Most commonly used with fluorescent microscopes but stained hair samples on a standard microscope can be illuminated with a Wood’s lamp to detect dermatophytes

depending on your experience and the equipment available. It is also important that all equipment is maintained properly and that quality control is conducted where appropriate (e.g. haematology and biochemistry analysers).

There are some tests – including serology, endocrine assays, microbial culture and sensitivity, and histopathology – that can only be performed in specialised laboratories. Most reputable laboratories perform these tests to a high standard and are open about their quality assurance measures. It is useful to develop a relationship with your laboratory; you will come to be familiar with their format and results and they will know what to expect from you. It also makes it easier to call in for advice – many laboratories now also have a network of clinical specialists that can give you advice on particular cases and results. It is also perfectly appropriate to use different laboratories for different tests, depending on their field of expertise, e.g. haematology and biochemistry, serological assays, endocrine assays, histopathology etc.

Dermatohistopathology, in particular, is becoming an increasingly specialised field and it is crucial that biopsy specimens go to a pathologist with a particular interest in skin diseases and who is clinically up to date. Many skin conditions can only be confirmed on histopathology and a number of new conditions are described each year. Some of these may be quite rare, leaving the pathologist in the ideal position to collate and disseminate information from their clinical colleagues.

#### How to Get the Best From an External Laboratory

- Take the appropriate samples. The bulk of this book is concerned with this, but check with the laboratory first if you are unsure. Some samples, for instance, may need special treatment or storage before despatch.
- Fill the submission forms in correctly, concisely and accurately. The amount of information will vary depending on the sample, but identification, age, sex and breed should be a minimum. Objective tests (e.g. blood tests) will require little else, but more clinical information can help the laboratory staff interpret the results.
- Skin biopsy specimens should be sent with a complete history, description of the clinical signs and differential diagnosis list. It can also be very helpful to attach photographs (it is very easy now to submit digital images by email) of the lesions. Each specimen should be submitted in a separate pot, clearly labelled with the biopsy site, to help match the histopathology to the clinical lesions.
- Package and label the samples correctly. You must ensure that legal requirements for posting pathological specimens are adhered to. Most laboratories now provide appropriate packaging and mailing containers, often with prepaid postage or a courier service. Guidelines for regulations in the UK can be found on: <http://www.royalmail.com/sites/default/files/Guidance-Document-Infectious-Substances-171012.pdf>.
- Always consider test results in light of the history and clinical signs. If they do not fit, then discuss them with the laboratory. It may be necessary to re-evaluate the clinical data, test results or re-submit samples, but an incorrect diagnosis can lead to inappropriate and potentially life-threatening treatment. Very few diagnoses come out of the blue – virtually all rely on clinicopathological correlation of all the available data. Remember to use your clinical skills, and make the diagnosis fit the data – do not twist the findings to justify a diagnosis.

## Sensitivity, Specificity, and Positive and Negative Predictive Value

Many journal articles and textbooks will refer to these statistical terms when referring to diagnostic tests. They can be qualified in absolute terms if figures are known (e.g. a test may have a sensitivity of 83%) or estimated in more relative terms (e.g. a test is highly sensitive but poorly specific). They describe the reliability with which tests can be used to confirm or rule out particular conditions. It is therefore important that clinicians have some understanding of these concepts when considering test results or trying to interpret published papers etc.

**Sensitivity** refers to the probability that a test will be positive if an animal has the condition tested for – in other words, how likely are false-negative results? **Specificity** refers to the probability that if animal does not have the disease, the test will be negative – in other words, how likely are false-positive results? An ideal test would have 100% sensitivity and specificity, but in practice virtually all tests will have some false-negative and false-positive results. An example of this would be using skin scrapes to detect *Sarcoptes* mites: if you find mites, then you can be nearly 100% sure that the animal has scabies, i.e. skin scrapes are highly specific for *Sarcoptes* infestation – there are no false-positives. Negative skin scrapes on the other hand do not rule out the possibility of scabies as you will not find mites in a large proportion of affected animals; i.e. skin scrapes are poorly sensitive for *Sarcoptes* infestations – there are many false-negatives.

The predictive values refer to the proportion of individuals for whom the test result is true. The **positive predictive value** (PPV) is the proportion of animals with a positive test that actually have the condition – i.e. that are correctly diagnosed. Conversely, the **negative predictive value** (NPV) is the proportion of animals with a negative test that do not have the condition.

The difference between these two sets of figures is that the sensitivity and specificity refer to inherent properties or accuracy of the test, whereas the PPV and the NPV give you some idea of the clinical usefulness of the test in a defined population that is tested. This is only useful, however, if the prevalence of the condition in the population is known as this has an effect on predictive values. For this reason, the sensitivity and specificity are more commonly quoted.

		Presence of the condition		
		Positive	Negative	
Test result	Positive	True positive	False positive	PPV
	Negative	False negative	True negative	NPV
		Sensitivity	Specificity	

Sensitivity = true positive / (true positive + false negative)

Specificity = true negative / (true negative + false positive)

PPV = true positive / (true positive + false positive)

NPV = true negative / (true negative + false negative)

### Worked Example

Let us say that 100 dogs with pruritus have a serology test for *Sarcoptes* performed. Of these, 10 dogs have *Sarcoptes*: nine are positive on an enzyme-linked immunosorbent

assay (ELISA) and one is negative. Of the remaining 90 dogs with another diagnosis, five are also positive on the ELISA.

		Presence of the condition		
		Positive	Negative	
Test result	Positive	9	5	PPV
	Negative	1	85	NPV
		Sensitivity	Specificity	

$$\text{Sensitivity} = 9/(9 + 1) = 90\%$$

$$\text{Specificity} = 85/(85 + 5) = 94\%$$

$$\text{PPV} = 9/(9 + 5) = 64\%$$

$$\text{NPV} = 85/(85 + 1) = 99\%$$

The test has a high sensitivity detecting 90% of *Sarcoptes* cases, and is highly specific, as 94% of animals with other dermatoses had a negative test. **In this population** of 100 tested animals the PPV is 64%, meaning that approximately two thirds of animals with a positive test have *Sarcoptes*, and the NPV is 99%, virtually excluding *Sarcoptes* in animals with a negative ELISA. In this example, the prevalence of *Sarcoptes* in the population is 10%. See how changing the prevalence (but keeping the sensitivity and specificity) affects the PPV and NPV. The PPV, in particular, can be poor for conditions with low prevalences, and, where possible, positive results for rare diseases should be confirmed with an alternative test.

When considering sensitivity and specificity it is also important to understand why the test has been used, the clinical presentation and what the expected outcome is. For example, urinary cortisol:creatinine ratios (UCCR) are quick, cheap and easy tests that can be used to diagnose canine hyperadrenocorticism (HAC). They are highly sensitive, as virtually all dogs with HAC have an elevated UCCR, but poorly specific, as UCCRs are also elevated in many other conditions. The diagnostic value in sick, polydipsic/polyuric animals can therefore be low; the UCCR is often elevated, but this can be of little clinical significance. It can, however, be a useful test in an otherwise healthy animal with alopecia, as a negative test will effectively eliminate HAC.

Before considering which test to perform, it is, therefore, important to think carefully about which differential diseases need to be investigated and which questions the clinician would like to have answered. Without careful consideration of these factors, the tests cannot give useful answers and this can effectively cost a lot of the client's money without reaching a diagnosis, which is most frustrating both for the attending vet and client. Although packages offered by commercial laboratories can be useful in some cases, using them without reflection is probably not the most effective use of the resources available to manage any given case.

