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Essential Practice Lab Tips, Tricks and Techniques Mini Series

Session Two: Urine Analysis: Not Just Any Old Jam Jar!

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This is often overlooked as a cheap and valuable source of clinical information and can be essential when diagnosing certain diseases such as renal failure, screening for conditions or monitoring therapeutic intervention. As with all other tests and fluids, it is open to abuse and misuse. In house analysis or preparation though is often crucial, as many substances are unstable or change in urine and cells quickly degenerate. Submission to external labs is still practical and feasible, but certain information can be lost and minimal processing in house may be required.

Sampling:

Four methods are available: free catch, manual compression, catheterised and cystocentesis, each of which has certain limitations and uses. Ideally, as occurs in human medicine, to maximise the information gathered we would collect all the urine produced in a 24 hour period. This is rarely if ever done in veterinary species, primarily due to practicality (and cost). We rely on a single urine sample as a 'snapshot' into disease processes. Urine though is constantly changing in composition as the disease changes, kidneys eliminate waste products and conserve water. Therefore to maximise the information we can gather **it is generally best to use an early morning sample** that reflects all the urine produced overnight and reduces hour to hour variation that occurs otherwise. It is also the most concentrated and acidic which helps to assess renal function and preserve proteinaceous structures. However, as this urine sample is stored longest *in vivo*, cellular degeneration will be greatest which can hamper sediment and cellular interpretation.

Before deciding on the urine sample timing and collection method, it is best to ask yourself what you are trying to identify or diagnose. Alternatively, serial or multiple urine samples may be required for accurate interpretation.

Samples should be clearly labelled with a form of identification, time of collection and the method of collection. This allows later analysis and interpretation to be as accurate and confident as possible.

Free catch / voided:

This is a good starting point for urine analysis as it reflects the entire urogenital tract. It is though very prone to sample contamination unless performed correctly. The animal may also not want to pee on demand. **It is not (or very rarely) suitable for bacterial culture and sensitivity.** The following are some guidelines that are recommended:

- ideally **use a designated sterile collection vessel (*e.g.* 'Uripet')** as this minimises debris, bacterial and chemical contamination. 'Kidney' or similar dishes can be used presuming they are cleaned and then rinsed **thoroughly**. Many chemical detergents and cleaning agents (*e.g.* chlorhexidine and hydrogen peroxide) will leave residues that can produce artefacts, especially on dipstick tests. Use of 'any old' container, such as an old tablet pot, is not recommended unless you know how it has been

handled, what it stored and have detailed knowledge of drug effects *etc.* on your dipsticks etc.

- midstream sampling is best as again this minimises skin, debris and bacterial contamination from the external genitalia.

Manual compression:

This is often performed opportunistically when the bladder is being expressed therapeutically or if indicated during general anaesthesia/sedation. Collection principles are identical to those for voided samples. This method though carries minimal risk of manual bladder rupture in certain situations. It is often slightly traumatic and therefore introduces haematuric (red cell) contamination. If urinary tract infection is present, compressive forces can reflux bacteria and inflammation into the ureters, upper urinary structures or prostate gland. This risk may be more theoretical than genuine, especially with regard to the prostate, as in male dogs, lower tract urinary tract inflammation or infection frequently involves the prostate anyway.

Catheterisation:

This is possibly the least common method performed and should be avoided in animals at risk of lower urinary tract infection, such as in endocrine disease and chronic renal failure. It is a skilled procedure, especially in females, and will commonly be traumatic and introduce haematuric contamination, especially if operators are unskilled. The catheter should be sterile, and the procedure performed in a sterile fashion. If done correctly, the sample can be used for bacterial culture and sensitivity. Excessive use of sterilising solutions such as chlorhexidine should be avoided as these will introduce artefacts, kill bacteria and potentially irritate the lining of the genital tract.

Cystocentesis

This is the ideal and only method recommended for bacterial culture and sensitivity. Digital immobilisation of the bladder allows urine to be aspirated by a needle and syringe introduced at a 45° angle through the ventral or ventrolateral wall of the bladder. Blind sampling is not recommended. This method will nearly always, to varying degrees, introduce haematuric contamination, even if the sample appears ‘clean’ grossly. As a screening method of collection and analysis, it may not be recommended unless your technique is consistently clean and atraumatic. However, it can be useful in combination with voided samples to localise the source of haematuria, pyuria (white cells) *etc.* It is ideal for bacterial culture, especially to confirm resolution of bacterial infection following antibiotics. It requires a sufficiently large bladder and patient co-operation (unless sedation is used).

Specimen preservation:

Typically fresh urine is collected into a sterile universal container and should be analysed within 1-2 hours of collection. This is the ideal situation and is recommended to maximise the amount and reliability of information obtained. However, preservation is often required if the sample cannot be analysed in this timescale or it is sent to an external lab. Because the composition of urine is very variable, saying how long it can be stored for and still be analysed accurately is not possible and will also vary depending on the analyte of interest. For example, the pH can increase dramatically due to loss of carbon dioxide and production of ammonia by certain bacteria. Generally, if the urine is alkaline, dilute and stored at high temperature the changes and artefacts induced, especially cellular, will be greatest. Some argue that this is not often clinically significant, as cellularity and features are often great enough to withstand prolonged storage. Subtle features though are likely to be lost and interpretation of the urine results may therefore be less useful or confident.

Refridgeration is always recommended, and arguably this allows confident examination up to 6-8 hours after collection. Postal samples should always be refridgerated until they are actually posted. Postal specimens are usually sterile universal containers. Various preservative tubes and substances are available and can be used, but each has problems and is really designed to stabilise specific urine substances or features. For example, formaldehyde (one drop of 40% formalin in 30 ml urine) or commercial “Mucolexx”is very good for cells and casts, but alters many dipstick tests such as glucose. A number of preservatives and urine specimens would therefore be required to fully stabilise all urine substances adequately. This is not feasible or common practice in veterinary medicine. One preservative that is commonly used is boric acid. **Boric acid preserved or transport swabbed samples are recommended when posting for bacterial culture and sensitivity**, as sterile non preserved containers will typically allow too much bacterial overgrowth for any useful interpretation. However, **these tubes must be filled to the correct level,** as too much boric acid relative to urine can prevent bacterial growth when cultured at the external lab. It can then create a false negative and ‘kill’ low numbers of bacteria. A sterile cystocentesis sample may then be best put in a plain pot only. Contact your laboratory to check what they advise. At a concentration of 0.8%, boric acid is also a good preservative for other urinary features, and apart from the pH, should not interfere with dipstick analysis. Only about 1 ml urine is required for standard full urine analysis. Ideally the sterile pot should be filled appropriately to prevent in vitro evaporation and changes in concentration.

Analysis:

If the sample has been refridgerated, it must be left to return to room temperature (about 10-20 minutes) before any analysis is performed. If analysed when cold, crystal formation and appearance is altered and many dipstick tests (which are enzymatic) may give falsely low results. Analysis involves gross examination, dipstick tests, measuring specific

gravity and sediment examination. Initially all these should be performed, but further serial monitoring may require only selected components.

Gross:

The gross appearance should be noted (after gentle resuspension if stored), as it provides valuable information. For example, grossly turbid samples may not benefit from centrifugation and concentration, visible haematuria can falsely elevate the protein levels and alongside marked colouration, can hamper colour examination of dipstick reactions.

Dipstick:

Always follow the specific timings and instructions for any dipstick strip as the majority are enzyme based and therefore time (and temperature) sensitive. A large number of dipstick test strips are available. These are generally similar in the substances measured, but vary in the pad reaction method, and are therefore subject to specific false positive and negative reactions due to artefactual interference. Some of these can be found in the accompanying data sheet, but as many are human based, certain veterinary idiosyncracies can also be seen. For example, whilst there is overlap in the pH of human and canine/feline urine, animal urine can be significantly more alkaline, in part because of delayed analysis commonly. If the pH is greater than 8-9, it exceeds the buffering capacity of the pads and causes false positive reactions, especially in the protein pad. If very alkaline urine is seen, but significant proteinuria is suspected, confirmation via alternative tests, such as a urine protein:creatinine ratio is recommended. Contamination with detergents can produce false positives, such as hydrogen peroxide and haemoglobin pads or chlorhexidine and protein pads. Administered or therapeutic drug effects are often poorly known, but some are. Cephalosporins (*e.g.* Ceporex) can create a false positive glucose dipstick reaction for instance and gapapentin a false positive protein reaction (also on analyser protein creatinine ratios (unpublished personal data)).

If artefact or contamination is suspected, consider rechecking on a fresh sample, using a different method and/or external lab for confirmation.

Some dipstick strips have a leukocyte pad. This is based on the esterase activity in leukocytes, primarily neutrophils, that is linked enzymatically to produce a purple colour. Whilst this is useful in humans, **it is unreliable in most veterinary species and should never be used alone to identify pyuria (increased white cells) and infection**. In dogs, it is insensitive, with microscopic evidence of infection/pyuria being seen when the dipstick remains negative. In cats, the opposite is true, with mostly false positives. When the pad turns purple there is no microscopic evidence of pyuria or bacteria cultured.

If the urine is grossly turbid or haematuric, it is advisable to allow the cells and debris to sediment and then dipstick the urine, or centrifuge the sample for sediment analysis and then analyse the supernatant.

Specific Gravity (SG):

Dipstick SG pads are not reliable and should never replace the use of a refractometer. SG measurement is based on the 'bending' of light as it passes through urine depending upon the concentration of substances dissolved in it. Measurement is made on a refractometer. Cells in the urine have no significant effect on the SG but the temperature of the liquid does. Refractometers are often designed to work in a specific temperature range that should be adhered to. Others can be adjusted as required. Specific veterinary refractometers are available (*e.g.* Misco Products Division, Cleveland) and possibly recommended as there is a slightly different feline scale relative to dogs. The clinical significance of this though in many situations is questionable, but subtle changes will be missed. Typically standard refractometers will over estimate cat urine SG. A correction formula is given below:

Feline SG = (0.846 x reading) + 0.154

All refractometers should be calibrated regularly with at least one liquid of known SG. Distilled water is the easiest liquid to use and should measure 1.000 on the SG scale if the refractometer is calibrated correctly. Poor cleaning and maintenance of the refractometer will allow gradual bias to be introduced otherwise.

Sediment analysis:

This is the final analytical step that is often not performed although quick and relatively straight forward. A significant and common problem is a variable approach and method, so results and analysis are not standardised so you cannot compare from person to person or over time. The following is one way of standardising analysis:

1. Thoroughly mix the specimen and transfer a specific amount, say 0.5ml, into a conical centrifuge tube or Eppendorf.
2. Centrifuge at low speed for 5 minutes. The exact speed (rpm) will vary on the size of centrifuge but can be calculated (see appendix). The centrifugal force should be about 450g, which commonly requires a speed of 1500-2000 rpm.
3. Remove the supernatant, leaving a standard volume in the bottom of the tube. For example, 0.2 ml.
4. Resuspend the sediment or pellet (which may not be visible) gently by flicking with your finger or 'racking' the bottom of the tube.
5. Using a standard (fine tipped) pipette, transfer a drop of this suspension onto a microscope slide and cover with a coverslip.

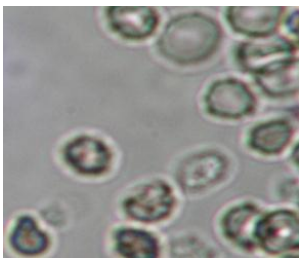
6. Examine under the microscope and semi-quantify results by grading or counting crystals *etc.* per x 10-20 low power field (lpf), with red and white cells only at high power (x40, hpf). See appendix for setting up the microscope for urine examination.
7. If required, an air dried preparation can be made using the line concentration method outlined. This is then stained as for cytology with a 'Diff Quik' rapid type stain in house.

Semi-quantitative grading can simply be 'occasional, mild, moderate and marked', but should be consistent from person to person. This quantification can be aided by the use of standardised wells that contain a specific volume of urine and grid system (*e.g.* Petstix). Microscopic examination can be performed unstained, but many prefer the use of stains such as "Sedistain". These can be very useful, but care should be taken to use standard amounts again so that variable dilution is not introduced. Also, stain precipitate is often misinterpreted as bacteria or the stain itself can become contaminated with bacteria over time. Both an unstained and stained wet coverslip preparation can be examined on the same slide if desired. If the sample is grossly haematuric, standard saline dilution or 2% acetic acid can be added to an aliquot or portion of the sediment (which lyses red cells) to allow white cells and epithelial cells to be examined. However in these situations, examination of an air dried preparation is often as rewarding.

Sediment is examined for red cells, white cells, crystals, epithelial cells, casts and bacteria. Other structures that can be seen include parasitic eggs, sperm and fungi. Cell numbers are commonly quoted as being normal when <5/hpf, although accurate and consistent reference values are difficult to obtain. Other structures are usually assessed and graded at low power (x10). The following is a brief description of the **unstained** appearance of some of these structures.

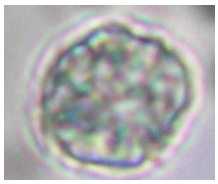
Red cells:

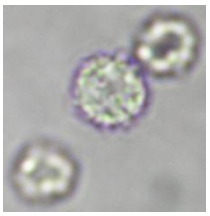
These are smaller than white cells, typically having an irregular circular outline with a central ring. They have no internal structures and therefore often appear smooth internally. Their shape though varies with the pH and specific gravity, and they can lyse if exposed to alkaline dilute urine for prolonged periods. They are often confused with fat droplets, yeasts or air bubbles. Red cells though are similar in size to each other compared to fat droplets. Fat droplets also appear slightly greener, very smooth and more refractile, typically being in a different plane/level within the coverslipped urine.



White cells:

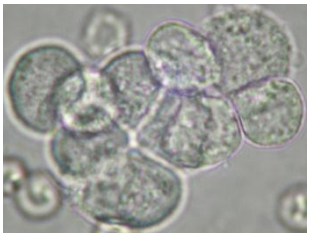
These are slightly larger than red cells (1.5-2 times) but are also circular. Distinctly though, they have internal structures and a nucleus, and thus within the circle there is a granular or grainy appearance and if variably focussed, the nucleus can usually be discerned. Again their shape is influenced by the SG (dilution), pH and also bacterial effects with infections. They can occur singly or in clumps. When clumped, each cell circle can still be traced completely.





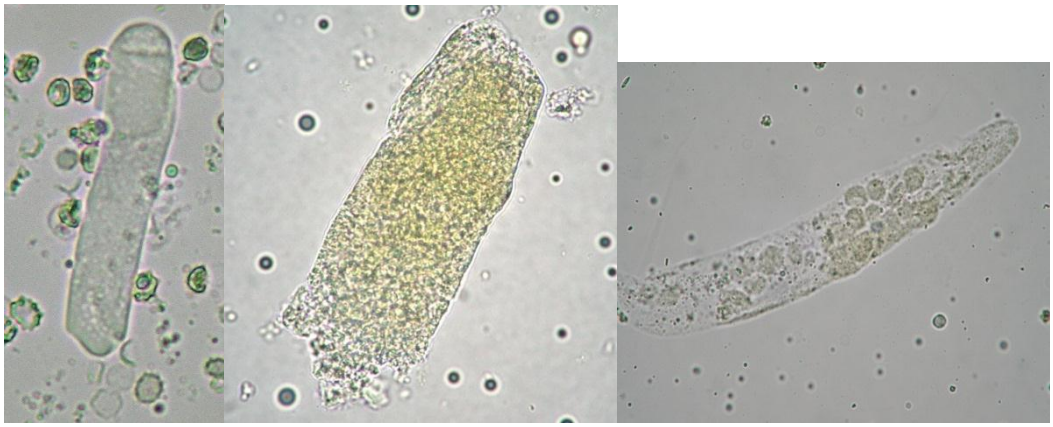
Epithelial cells:

These are typically the largest cells seen, but size is very variable as various types are seen depending on the sample type; from basal, transitional to squamous. Commonly they are round with a grainy internal appearance. However, the nucleus is relatively small within the cytoplasm, compared to white cells. Squamous cells can appear more angular and thinner, with or without small nuclei. Cohesive groups can be seen where the boundary from one cell to the next is lost or is seen as a straight line.



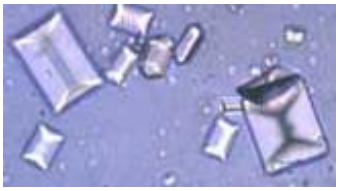
Casts:

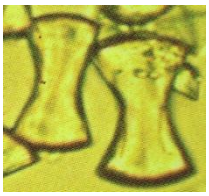
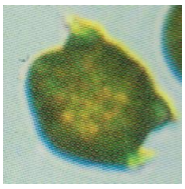
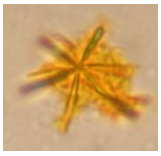
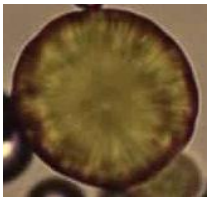
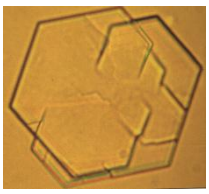
These are primarily urinary mucoprotein (Tamm Horsfall) that originates in the kidney tubules. Within this, cells or other material such as fat can be embedded. The key feature is their cylindrical or tube-like appearance which has parallel sides with a thicker centre. Over time, casts can change, with for example hyaline casts transforming into waxy casts. They are classified according to the dominant appearance or component. For example hyaline casts are pure mucoprotein, with epithelial casts over time degenerating into granular casts when no distinct cell shapes can be seen. Red, white or mixed cell casts can also be seen.



Crystals:

These are best assessed fresh and in wet mounted preparations. Whilst they can be visible in air dried smears, their shape and nature often changes and specific types cannot be identified. The actual formation of crystals is very complex (so called 'crystal habit') and whilst certain types are commonly known and seen, each 'type' can have several different forms depending upon the prevailing conditions when they form. Mixed 'types', such as struvite and oxalate, can therefore also be seen. Some can occur in healthy dogs and cats without clinical signs/disease, especially as they can precipitate or form *in vitro*. The nature or type of crystals does not necessarily correlate with the type of any urolith present. Essentially their presence supports a risk, but significance must be interpreted alongside clinical signs, imaging and the urinary pH.

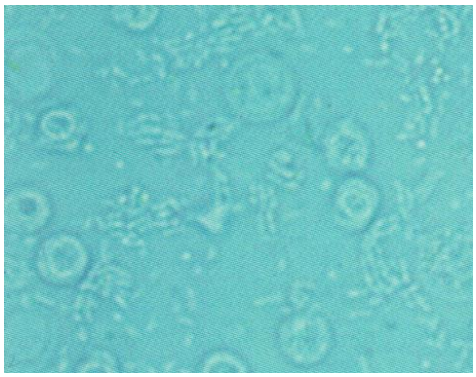


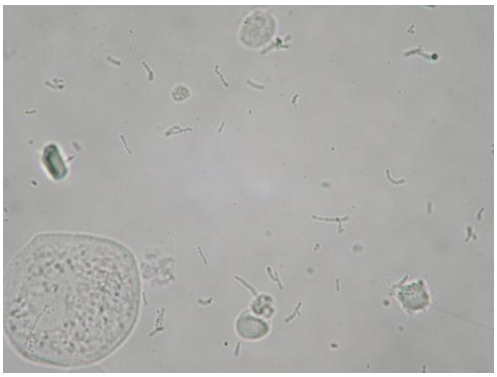






Bacteria:

These are typically seen using either x40, or even oil immersion (x100). A significant number (greater than 10-100,000) is required before these are able to be seen, and thus urine culture is always recommended to exclude infection more definitively. They are often confused with amorphous debris that is moving randomly under Brownian motion. Shapes should appear uniform and consistent, often being present in clumps.

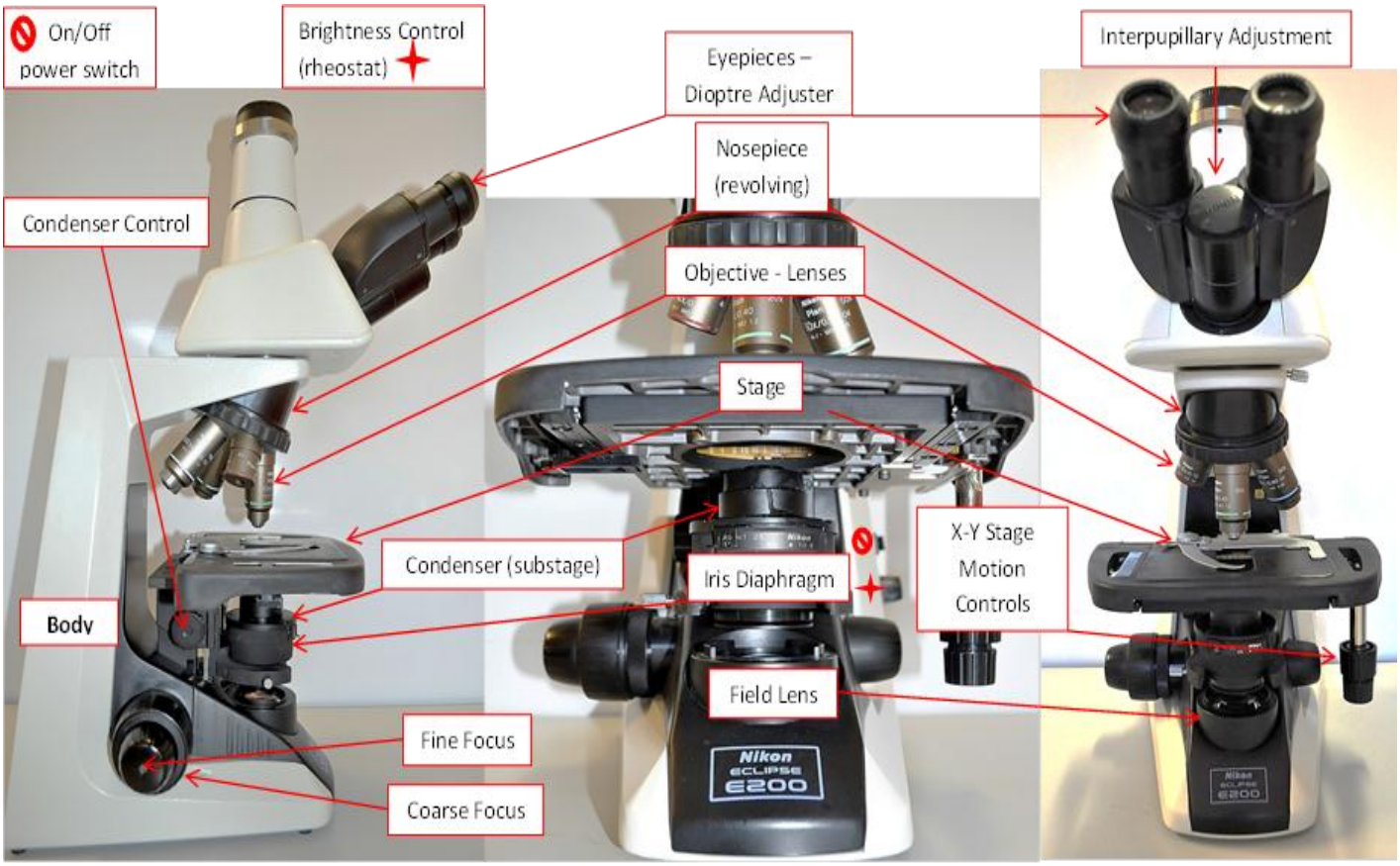
If in doubt as to the identity of any urine structures, using stains such as Sedistain, 0.5% toluidine blue or new methylene blue is recommended. Alternatively air dried films and 'Diff Quik' stained preparations should be made and examined before diagnosing specific structures.





Appendix

Setting up a microscope:



Microscopes without an adjustable field iris diaphragm

1. Turn the lamp on, (adjust the brightness for comfort) and adjust the interpupillary distance so only one image is seen.
2. Place a coverslipped slide onto the microscope stage.
3. Using the x10 objective lens, focus on the slide material. Ensure the eyepiece focus settings are equal and set at 'baseline'.
4. Using the x40 lens, focus on the slide material. Then change back to the x10 lens, and using only your right eye, change the eyepiece focus settings until the image is focussed. Do **not** use the fine/coarse focus control knob. Repeat this procedure using only your left eye.
5. Repeat step 4 using the x40 lens to ensure the eyepiece focuses are perfectly set for your eyes.
6. Set the condenser to just below its highest setting using its focus control knob.
7. Use the desired lens for examination. If the microscope has an adjustable aperture diaphragm on the condenser, this should be set to the same figure as the magnification of the lens (*e.g.* 4, 10, 40 or 100). This aperture should be changed each time the objective lens is changed ideally. If not, a compromise setting for most objective lenses is 40.

The above guidelines allow each microscope to be used to the best of it ability. They do not have to be adhered to, but if not, image quality will be lost and this may hamper examination of material. Once they have been followed once, only steps 1-5 need to be repeated for the microscope to be adjusted to each user, because each person has slightly different strength eyes and interpupillary distances.

The above settings are designed to optimise the microscope for examining cells and cytological preparations. **However, when examining unstained fresh urine or scrapes *etc* for parasites, it is advisable to lower the condenser to its lowest setting and close the field iris diaphragm down**. This may require increasing the brightness, but will maximise the refraction and therefore make sediment and parasites very easy to see.

Centrifugal Force:

The following equation allows the speed (required) to be calculated to give a certain centrifugal force.

Centrifugal force (g) = $1.118 \times 10^{-5} \times R\ (cm) \times RPM$

R is the radius of the centrifuge arm in cm.
RPM is the revolutions per minute or speed required when spinning samples.