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Uranotest
Slide staining set

For veterinary use only

The Uranotest® slide staining set is a kit adapted to the needs of veterinary clinics, developed to facilitate stain tests carried out at the clinic by making them easier.

Cytology tests are becoming increasingly commonplace in clinical practice. However, incorrect sample taking or processing can prevent a correct diagnosis.

The Uranotest® kit has been designed to make the vet's work easier and to minimise many of the common mistakes made in daily practice.

System components

The set comprises 4 elements:

1. Staining jars with lids

The set contains 5 jars with a 25 ml capacity, designed to contain the liquids commonly used for the various tests. Being the jar with the green lid, the specific for the fixing solution.

2. Support base

Serves as the support for the staining jars and prevents involuntary spills during handling.

3. Slide rack

Allows for easy handling of up to 3 slides at the same time.

4. Adhesive work mat

A large work mat is included, measuring 40 cm x 23.5 cm made of easy-to-clean vinyl material which adheres to the work desk for increased stability.

Proper staining procedure

The set has been designed for use with Romanowsky or Diff-Quik®-type stains. These stains consist of a first solution with methanol (fixing solution), a red dye solution (eosin) and a blue dye solution (methylene blue).

Staining times vary a lot according to the references consulted and sample type (thickness, nature, proteins, etc.). Our advice is to use the time guidelines set out below, unless the samples are very thick, and to always carry out a visual check that the sample acquires the required colouring:

Before use

1- Remove the protective paper from the work mat and adhere it to the place of work. The mat can be easily removed and placed in in a different location later.

2- Place the jars in the support base and place the assembled unit on the work mat.

3- Fill the jars with the solutions to half their capacity (approximately 12.5 ml).

Jar 1: fixing solution (green lid)
Jar 2: water (preferably distilled)
Jar 3: dye solution 1 (red)
Jar 4: water (preferably distilled)
Jar 5: dye solution 1 (blue)

4- The set is ready to use.

Staining procedure

5- Leave the slides to dry with the sample exposed to the air, without using any paper or fabric to avoid introducing foreign particles or "artifacts".

6- Place the slide or slides (up to 3) in the slide rack.

7- Immerse the rack in jar 1 (fixing solution; green lid) for 5-10 seconds. Remove and allow to drain.

8- Immerse the rack in jar 2 (red dye solution) for 5-10 seconds. Remove and allow to drain.

9- Immerse the rack in jar 3 (water) for at least 15 seconds as an intermediate step for washing the sample and to avoid mixing the red and blue solutions. Depending on the vet's preferences, the slides can be immersed straight from the red dye into the blue one without previously washing in water.

10- Immerse the rack in jar 4 (blue dye solution) for 5-10 seconds. Remove and allow to drain.

11- Wash the sample again in jar 5 (water) for 15 seconds.

12- After this step, some vets prefer to carry out an additional wash with distilled water to completely eliminate any dye remains.

When old, mixed or contaminated dyes are used, the staining times must be lengthened. However, the use of new dyes or dyes that have not been used very much will improve the quality of the stains.

> The Uranotest® stains kit can be used with any brand of staining liquids. However, the staining times recommended here are established for use with the Uranotest® staining liquids.

Main errors made in staining procedures

1- Use of old liquids which may contain dye precipitates or bacterial overgrowth through evaporation of the dyes' alcohol component.

2- Use of liquids which have been previously used for several stain tests, with the ensuing appearance of cellularity from samples that do not belong to the one being stained.

3- Use of inadequate large-sized containers which may need to be filled with a large quantity of fixing agent and dye which means that, to avoid financial losses, they are not replaced with the required frequency.

4- Use of opaque containers that do not allow the suitability of the dyes being used to be appreciated

5- Inadequate obtainment or processing of the sample (see section on advice for obtaining a good sample).

In general, the liquids ought to be changed at least every week (in the case of water daily or, even before, if several different samples have been used in a short time.

This recommendation is applicable to the use of liquids of any commercial brand, not only the Uranotest® brand.

Main advantages of using the Uranotest® slide staining set

1- The jars have a capacity of 25 ml. When half full with a volume of just 12-15 ml it is possible to stain samples, allowing for a much more frequent renewal of the liquids without incurring in financial losses.

2- The jars have a lid to minimise the risks of contamination and evaporation of the liquids.

3- The adhesive mat makes it possible to establish a comfortable work area that is easy to clean and that protects the clinic's and/or laboratory's furniture against soiling.

4- The staining system has a solid and firm base in which the jars can be placed, avoiding spills through use of inadequate jars, designed for other purposes and sometimes used for staining.

5- The transparent jars make it easy to check that the liquids are always clean, free of artifacts, sediments or precipitates.

6- The staining rack allows for easy handling of up to 3 slides, without staining the fingers with the dyes and facilitating complete immersion in the fixing solution, the dye solutions and the wash water.

7- The fixing solution and dye solutions 1 and 2 are sold individually. Bearing in mind that the fixing solution tends to evaporate and is also used to fix samples for sending to a laboratory, normally a greater quantity is used than in the case of the dyes. Many companies oblige you to purchase the full kit with the fixing agent and the two dyes, leading to unnecessary losses.

Advice for obtaining a good sample

- Materials to be used for an appropriate puncture

The pipetting needles normally used range from 20 to 25G, mainly 23G (blue), although the 20G (yellow) or 21G (green) are used increasingly, and also produce very representative samples.

These pipettes can be attached to syringes that range from 5 to 20 ml (normally 10 ml). When aspiration is applied during puncture, it will never be greater than three quarters of the volume of the syringe, and will take no more than 2 to 3 seconds, with a view to ensuring that the collected content does not penetrate the inside of the syringe and cannot be recovered.

The softer the tissue, the smaller the syringe and pipetting needle must be. In fragile samples or where cellular exfoliation is easy (lymph nodes, round cell tumours, endocrine cells, etc) it is advisable to puncture without aspiration, with a view to avoiding the contamination with blood of the sample and the appearance of cell artifacts due to the pressure exerted during aspiration with

the syringe.

- Sample-taking in specific locations

Skin masses

In small-sized masses the sample will be taken as close as possible to the centre of the injury. However, in large masses, it is preferable to take the sample from the periphery as the central part tends to be necrotized.

Both the fine-needle puncture (FNP) and fine-needle aspiration (FNA) techniques can be used. In the case of FNP, the needle will only be moved from front to back, and we will never change the direction of the needle inside the mass to minimise contamination with blood.

Surface injuries and ulcers

Normally, only inflammatory cells will be obtained (even though they are secondary) and it may be that sufficient cells are not exfoliated by imprint, therefore, whenever it is feasible, we must carry out FNP/FAP of the injury (beneath the ulcer) in addition to the smear.

The imprint tends to be useful to determine the presence of primary or secondary fungal or bacterial infections.

Scraping

This is mainly used for external injuries, although sometimes it is used for samples obtained during surgeries or necropsies. Their main drawback is the same as in the case of imprints, obtaining samples with surface inflammation or contamination. Their most frequent and most effective use is with lesions of the feline eosinophilic granuloma complex and dermatophytosis. When we scrape very dry surfaces, we must deepen sufficiently to obtain serum or blood, and thereby favour cell cohesion for then extending on the slide.

Swab

Mainly indicated for vaginal samples and samples of the outer ear canal, and also for fistulous tracts. If the sample is of dry injuries, we can moisten the swab with saline solution, but will not use lubricant gels. The swab stick is rolled rather than glided over the slide.

Lymph nodes

To take samples of this tissue, we never use aspiration, rather FNP and when spreading the sample it is not pressed, as these cells are very fragile and will break.

Intracavity structures

The following recommendations are for intracavity structures. With ultrasound guided punctures, take the precaution to remove all of the ultrasound gel before carrying out the puncture.

- **Liver:** carried out when there is hepatomegaly or echogenic alterations. Either FNP or FNA can be carried out, or both. The only precaution is to make sure that the platelets are normal.

- **Spleen:** in diffuse or localised illnesses (careful with cysts). Never carry out FNA, only FNP.

- **Páncreas:** if done quickly and only FNP, it will not provoke pancreatitis. Has great diagnostic value, fundamentally in critical mass.

- **Kidney:** only in masses or renomegaly. It is recommended to only do FNP and without moving the needle much. It is advisable to inform the owner that the patient could have provisional hematuria lasting a few days.

- **Bladder:** in masses or thickened mucous. Because urine is an irritant, it is better, as a step prior to the cytology to carry out washes of the bladder using serum. We can do FNA, but it is preferable to carry out traumatic catheterisation, as dissemination of the disease through the puncture pathway has been described.

- **Prostate:** when there is heterogeneity, masses or cysts. Either FNP or FNA can be carried out. In cysts, it is recommended to ensure aspiration of all of the liquid.

Liquids

Some liquids have the drawback of degenerating very quickly, in a period of less than 2 hours (cerebrospinal fluid, urines, liquids with a low protein content, etc.). It is advisable to always carry out extensions (cell morphology is preserved better) and, whenever possible, to place the remaining liquid in EDTA (improves conservation).

In liquids with a low protein content to keep the cells better for longer a few drops of autologous serum (from the patient) can be added, as the proteins stabilise the cell membranes.
When dealing with synovial fluid, a couple of drops of serum would not be necessary or such a quick processing, as this fluid in itself is very rich in protein. Ascitic and pleural fluids also have a high protein-content in many cases.

Cerebrospinal fluids and bronchoalveolar and tracheobronchial lavages are especially problematic. In the case of adding autologous serum, we must specify the quantity, as the cell count will vary.

Thyroids

Either FNP or FNA can be carried out, when there is an increase in size, cysts, or isolated masses.

Intrathoracic masses

The most frequent occurrence tends to be cranial mediastinal masses. FNP or FNA can be performed.

> For more information, see the documents prepared by the technical team of the Cytology and Pathological Anatomy department of Urano Vet, at www.uranovet.com

Products of the Uranotest® staining pack

Ref D248 - Uranotest® staining pack

- Support base
- 5 jars
- Slide rack
- Work mat
- Booklet with recommendations for correct staining

Ref D249 - Uranotest® starter pack

- 1 Uranotest® staining pack comprising a:
  - Support base
  - 5 jars
  - Slide rack
  - Work mat
  - Booklet with recommendations for correct staining
- 1 bottle of Uranotest® fixing solution 250 ml
- 1 bottle of Uranotest® staining solution 1 (red) 250 ml
- 1 bottle of Uranotest® staining solution 2 (blue) 250 ml

Ref D245 - Uranotest® fixing solution 250 ml

Ref D246 - Uranotest® staining solution 1 (red) 250 ml

Ref D247 - Uranotest® staining solution 2 (blue) 250 ml

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