Universidade de Trás-os-Montes e Alto Douro

Validation of fast-read microscopic slide on urine sediment examination of dogs and cats – a preliminary study

Master Thesis in Veterinary Medicine

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All information present on this study is the entire responsibility of the author.

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Abstract

Urinalysis is a diagnostic screening test that can diagnose and monitor genitourinary, kidney and metabolic diseases even before the onset of clinical signs. Although it is an extremely useful, non-invasive, readily available and inexpensive tool it is a much-neglected test in veterinary medicine because it's ponderous, time-consuming, imprecise and has wide variability.

This study evaluated the diagnostic accuracy and interchangeable capacity of the counting cell chamber Pentasquare slide in comparison with the traditional method of urine sediment using slide and coverslip. This study included 34 patients, 17 dogs and 17 cats, that were admitted in the veterinary hospital of the University of Trás-os-Montes e Alto Douro (HV-UTAD) between May 2nd and July 31st of 2019. The population under study had urinalysis performed whether because a genitourinary or kidney disease was suspected or to evaluate the evolution of a previous diagnosed pathology. To every patient a type II urinalysis was performed (dipsticks and urine sediment). In all cases sediment evaluation was made with 3 preparations: one traditional method for urine sediment preparation and two preparations on the counting cell chambers in study.

The statistical analysis of method comparison by using Passing-Bablock regression and Bland Altman plot demonstrated that the counting cell chambers can be used interchangeably with the slide and coverslip method. For qualitative parameters the sensitivity and specificity revealed high values, between 43% to 84% and 89% to 100% respectively. The agreement rate between methods was moderate to substantial (values from 0,47 to 0,77), assessed by the calculated Cohen's Kappa value. Besides these values that assess the capacity of the counting cell chamber to replace the traditional method, reproducibility and repeatability were also calculated. Repeatability revealed high values of correlation between observers, being the lowest value obtained of 68% and the highest of 100%. As for reproducibility the counting cell chambers revealed correlation values of 61% to 100% for different elements, except for calcium oxalate crystals.

Even though more studies are needed to evaluate the use of the Pentasquare slide as reference method for urine sediment in veterinary medicine this preliminary study verifies that counting cell chambers can be used for urine sediment evaluation in dogs and cats.

Keywords: urinalysis, sediment, microscopy, cell counting chamber

Resumo

A análise de urina é um exame complementar de diagnóstico capaz de diagnosticar e monitorizar patologias do sistema geniturinário, de origem renal ou metabólica, mesmo antes do aparecimento de sinais clínicos. Mesmo sendo um exame extremamente útil, não-invasivo, de disponibilidade imediata e pouco dispendioso é largamente negligenciado em medicina veterinária por ser considerado trabalhoso, demorado, impreciso e com grande variabilidade de resultados.

Este estudo avalia a precisão diagnóstica e capacidade de permutação da câmara de contagem de células – pentasquare – em comparação ao método tradicional de sedimento urinário usando a lâmina e lamela. Este estudo incluiu 34 pacientes, 17 cães e 17 gatos, que foram admitidos no Hospital Veterinário da Universidade de Trás-os-Montes e Alto Douro (HV-UTAD) entre maio e julho de 2019. O exame de urina foi realizado à população em estudo por suspeita de patologias renais ou geniturinárias ou como teste de controlo de resposta a tratamento. Em todos os animais foi realizada uma análise de urina tipo II (tira reativa e sedimento urinário). Em todos os casos o sedimento foi avaliado em três preparações: uma preparação no método tradicional para sedimento urinário e duas preparações nas câmaras de contagem de células em estudo.

A análise estatística de comparação de métodos realizada utilizando a regressão de Passing-Bablock e o gráfico de Bland Altman demonstrou que as câmaras de contagem de células podem ser permutadas pelo método tradicional de lâmina e lamela. Para os parâmetros qualitativos a sensibilidade e a especificidade revelaram valores altos, entre os 43% e a 84% e os 89% a 100% respetivamente. A taxa de concordância entre métodos, estimada pelo valor de Cohen's Kappa, foi de moderada a substancial, sendo o menor valor obtido de 0,47 e o maior de 0,77. Além dos valores calculados que estimam a capacidade de a câmara de células poder substituir o método tradicional, também os valores de reprodutibilidade e repetibilidade foram calculados. A repetibilidade revelou valores de correlação entre observadores elevados, de 68% a 100% para os diferentes elementos. A reprodutibilidade mostrou valores de correlação entre 61% a 100% para os diferentes elementos, com a exceção dos valores obtidos para o parâmetro dos cristais de oxalato de cálcio.

Ainda que mais estudos sejam necessários para avaliar o uso da câmara *pentasquare* como método de referência na avaliação do sedimento urinário em medicina veterinária, este estudo preliminar verifica que estas câmaras podem ser utilizadas para a análise de sedimento urinário em cães e gatos.

Palavras-chave: análise urina, sedimento, microscópio, câmara de contagem de células

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Abbreviations

% - percentage

>- higher

CI – confidence interval

CLSI – Clinical and Laboratory Standards Institute

cm – centimeter

dL – deciliter

ELISA – enzyme-linked immunosorbent assay

h – hour

HPF – high power field

HV-UTAD – Hospital Veterinário da Universidade de Trás-os-Montes e Alto Douro

L – liter

LPF – low power field

mg – milligram

mL – milliliter

mm – millimeter

nr – number

º - degree

ºC – celsius degrees

PNV – predictive negative value

PPV – predictive positive value

RBC – red blood cell

rpm – rotations per minute

RSS – urinary relative supersaturation

SG – specific gravity

spp – species

SQEP – squamous epitelial cell

SSA – sulfosalicylic acid

THM – Tamm-Horsfall mucoprotein

uL – microliter

USG – urinary specific gravity

WBC – white blood cell

I. Introduction

Urinalysis is a non-invasive test that should be performed as a general panel for every patient that comes into the clinic alongside with complete blood count and serum chemistry. Besides being a test that is easy and cheap to do, in most patients, the collection of urine is quite easy performed (Perazella, 2015).

A complete analysis of urine should be interpreted along with the main complaint for the office visit, clinical history of the patient and results of the remaining diagnostic tests. Urine physical, chemical and sediment analysis provides useful information, and can be of diagnostic use for nephropathies, genitourinary and metabolic diseases (Parrah *et al.*, 2013). Even more, the evaluation of urinary sediment can be used to make an early diagnosis of kidney disease before the onset of the pathology as well as serve as a monitoring test to assess the evolution of a disease or how the patient is responding to treatment (Chan and Szeto, 2004; Hernandez *et al.*, 2019).

Even though it is of extreme importance to perform a complete urinalysis, the urine sediment examination has suffered a progressive decline in the past decades (Fogazzi and Garigali, 2003) because the test is considered a time-consuming, laborious and imprecise procedure (Chien *et al.*, 2007). Thus, the need for an alternative to the traditional method of urine sediment examination is needed, in order to allow an easier urine sediment preparation, analysis, report and interpretation of results (Chan and Szeto, 2004). So, if the clinicians become more comfortable analyzing urine sediment and are able to gather more reliable information, urine analysis can be used once more as a regular diagnostic test and can be given the use that deserves (Verdesca *et al.*, 2007).

This study is developed to determine if the counting cell chamber pentasquare can be an alternative to traditional method of urine sediment since it seems offers a set of advantages over the traditional method to promote an everyday use of urine sediment by any clinician in every veterinary practice (F.L. Medical, 2009).

II. Literature Revision

1. The Kidneys, Urine formation and importance of urinalysis

Kidneys are the sum up of the action of each functional structure unit: the nephron. Receiving approximately 25% of the cardiac output (Reece, 2015) their primary function is to regulate the composition of the extracellular body fluid (Brown, 2011). Blood volume, extracellular fluid volume, systemic arterial blood pressure and hematocrit regulation are extremely influenced by kidney function as well as acid-base balance, plasma electrolytes, minerals and metabolites (Brown, 2011).

Urine formation happens due to various mechanisms being the major ones: glomerular filtration, tubular reabsorption and tubular secretion. Urine starts being formed in the glomerulus as an ultrafiltrate (Reece and Rowe, 2017) and becomes tubular fluid flowing throughout the tubular structures of the nephron due to hydrostatic pressure differences inside Bowman's capsule and renal pelvis, leading to pelvic urine accumulation. The urine is then transported to the bladder throughout peristaltic movements in the ureters. The ureters enter the bladder in an oblique angle forming a functional valve (ureterovesical valve), preventing a retrograde entrance of urine in the ureters allowing vesical filling (Reece, 2015).

Urinalysis becomes of major importance once it allows the detection of renal disease before kidney failure onset by evaluating the presence of casts, white and red blood cells and bacteria in urine. Some metabolic diseases can also be detected by urinalysis as diabetes mellitus and liver diseases by measuring glucose and bilirubin respectively, as well as intravascular hemolysis by evaluating the presence of hemoglobin in urine (Parrah *et al.*, 2013; Lee, Ha and Ryoo, 2016).

Urine analysis in veterinary medicine can be used as a diagnostic screening test, side by side with complete blood count and serum chemistry (Chien *et al.*, 2007). Collection of urine is, in most cases, a non-invasive procedure that offers a "liquid biopsy" of the kidney and the entire urinary tract (Yasui *et al.*, 1995). As well, is an important tool for evaluating a disease evolution or the patient response to an ongoing treatment (Cho *et al.*, 2019).

2. Collection of urine sample

Urine can be collected by different methods, which should always be identified once they can influence the interpretation of the results by the clinician (Chew *et al.*, 2011). All methods have advantages and disadvantages and should be chosen according with the patients' health state and the urinalysis goal.

2.1. Free-catch or voided urine

Natural micturition is the less invasive method to collect urine (Sink and Weinstein, 2012) and can be performed as an initial screen test or be made by the owners when assessing the evolution of a pathology by urinalysis. This is the method of choice to evaluate the presence of red blood cells in urine and is also the method of urine collection that carries less risks for the animal (Chew *et al.*, 2011).

The urine can be collected during normal voiding (**Figure 1**) or by manual external compression of the urinary bladder. In both the first stream should be discarded to reduce the contamination by bacteria, cells and other debris present in the urethra, vulva or prepuce (Reine and Langston, 2005). Urine collected by normal voiding can be performed both by the clinician or the tutor, although, a non-experienced person, can cause the animal to cease urinating in the attempt of collecting urine (Sink and Weinstein, 2012) and the chance of contamination from the environment or the container (**Figure 2**) is higher (Chew *et al.*, 2011). Manual compression of the urinary bladder, although convenient, should be made with care since it can cause trauma to the bladder, reflux of contaminated urine to the ureters, kidney and prostate and is a technic that can't be performed in patients following a cystotomy or in the postoperative period of a laparotomy. In overly distended urinary bladders manual compression should be made with extreme caution since it can cause rupture of the bladder. Using this technic in male cats can be harder not only due to the difficult handling of the animal, but also because of the difficulty in initiating voiding due to the small diameter of the urethra (Osborne and Stevens, 1999; Kouri *et al.*, 2000).

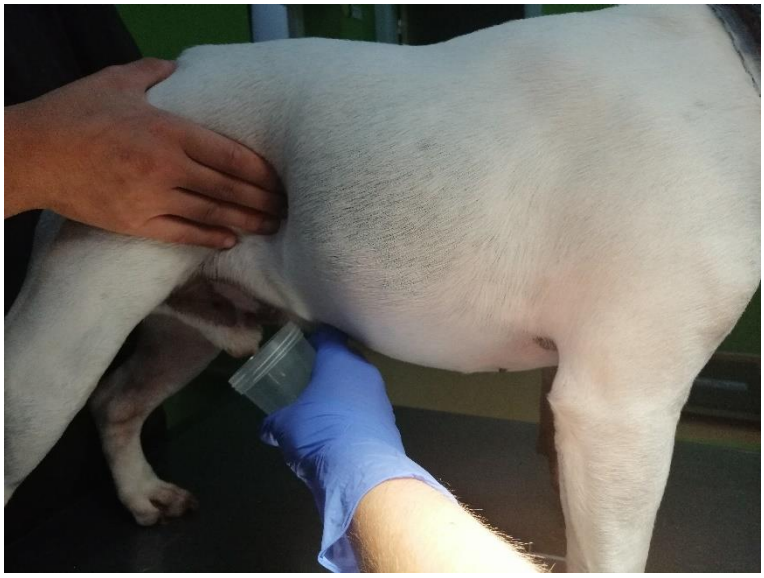

Figure 1 Manual expression of the urinary bladder of a male French Bulldog by compressing the bladder through the abdominal wall while standing and urine being collected to a sterile container for posterior urinalysis (author image)

Figure 2 Transparent urine container sealed. Transparent containers are preferable allowing the initial physical examination of urine without risking changes due to air exposition (author image)

2.2. Urinary Catheterization

Transurethral catheterization is a method of urine collection that requires trained personnel to perform it, can be used in either dogs or cats (Kouri *et al.*, 2000). Catheterization can be used in female and male dogs and cats, although the patients' handling depends on their species and sex. On male dogs, urine can be collected by catheterization with the patient awake, on the other hand most female dogs and both female and male cats need to be sedated. A sterile and careful technic should be used to ensure the integrity and health of the patients' urinary tract and that urinary bacterial cultures can be performed without the growth of contaminant bacteria (Sink and Weinstein, 2012). The catheterization allows collection of urine without the need for a distended bladder and it's not dependent on the animals' willingness to urinate (Rizzi *et al*., 2017).

Male dogs should be placed in lateral recumbency, although a standing position can also be taken in larger animals or when restraining the animal is stressful. Cleaning the surrounded area of the urethral orifice is essential by clipping longer hairs that might interfere and using water and disinfectant soap to wash the prepuce and exposed portion of the penis. All cleaning procedures should end with rinsing water to avoid contamination of the urine. The prepuce is retracted caudally to its junction with the abdominal wall exposing the penis that should be

pushed cranially (**Figure 3**). All the material used should be sterile: the gloves used by the operator, the lubricant used at the urethral orifice and the catheter itself. Within possibilities the prepuce should be kept retracted until the end of the procedure to minimize contaminations. After introducing the catheter, it should be advanced slowly and steady taking into account that resistance is expected while passing the base of the penis and the ischial arch (Reine and Langston, 2005).

In male cats the procedure stands on the same basis with some adjustments. Cats can be placed in lateral or dorsal recumbency, with the pelvic limbs pulled cranially and the tail pulled dorsal and cranially in order to obtain maximum exposure of the prepuce. The penis should be extended from the body by pushing the prepuce towards the body and cranially and retracting the (Rizzi *et al*., 2017) glans caudally in order to contradict the natural curvature of the penis and facilitate inserting the catheter (Chew *et al.*, 2011).

Figure 3 Penis exposure in a French Bulldog by retracting the preputial skin. In this patient there isn't need to clip the hairs. The penis and surrounding area are cleaned with an antiseptic solution for further catheterization. Note the error on the example figure by not using sterile gloves (author image)

Placing urethral catheters in female dogs can be performed with the animal in lateral or ventral recumbent position. The vulva and surrounding tissues should be cleaned with water and an antiseptic solution and, if needed, the surrounding hairs can be clipped, and long tails wrapped with adhesive. As in male animals the procedure should be performed in the most aseptic way possible. Sterile lubricant should be applied for the insertion of the otoscope. The otoscope speculum is inserted through the vulva and advanced along the dorsal wall of the vestibule until pass the clitoral fossa until it is possible to observe the urethral orifice at the urethral papilla. The lubricated sterile catheter is then inserted through the urethra and gently

advanced until urine starts to appear in the catheter, to which after only 1 or 2 centimeters (cm) should be advanced into the bladder. Some clinicians choose to perform transurethral catheterization in females by digital palpation. This technic consists in, while wearing sterile gloves, placing a finger in the vulva until the urethral papilla is felt and placing the sterile catheter in the urethral orifice by trial and error (**Figure 4**) (Kouri *et al.*, 2000).

Female cats are usually placed ventrally with the pelvic members alongside the body cranially and the tail retracted or wrapped with adhesive. The procedure to insert the urinary catheter is in all the rest similar to the one performed in female dogs, although, usually, more challenging (Rizzi *et al*., 2017).

In either technic, species or patient's sex, a sterile syringe or a sterile container can be placed at the end of the catheter to collect the urine. Passing the catheter through the urethra can cause some tissue trauma that will, naturally, increase the number of red blood cells, transitional and squamous cells in the urine sample, not being clinically relevant once it's an iatrogenic finding. Thus, this method of collection will change the number and quality of elements present in chemical and microscopic evaluation of urine sample, for which the clinician should be prepared (Alleman and Wamsley, 2017).

Figure 4 Demonstration of a transurethral catheterization of a female dog by digital palpation using a mannequin in ventral position. Using a sterile glove and sterile lubricant the clinician's finger is inserted through the vulva in an upward direction until the urethral papilla is felt. The urinary catheter is inserted through the vulva underneath the finger resting on the pelvic floor and into the urethral orifice (adapted from Rizzi *et al*, 2017)

2.3. Cystocentesis

Cystocentesis it's the method of choice for urine bacterial culture and can be performed without patients being sedated. The technic is roughly performed with the same principals between species and gender (Reine and Langston, 2005). The animal should lay on dorsal recumbency and the bladder should be located. In animals with long fur or who are soiled, a small area should be clipped. In either clipped or unclipped animals, the area of cystocentesis must be thoroughly cleaned. The bladder can be located by palpation of the caudal abdomen or by ultrasound, although a poorly filled bladder might be difficult to locate without ultrasound. A syringe of 6 to 12 mL attached to a 21to 23-gauge needle is placed and inserted into the abdominal wall at an oblique 45º angle being advanced caudally toward the pelvic inlet (**Figure 5)**. While aspirating urine to the syringe light digital pressure should be applied to the bladder, although with care not to risk leakage of urine around the needle site. The digital pressure over the bladder should be released prior to removal the needle (Sink and Weinstein, 2012).

Even though cystocentesis is considered a secure procedure, it has some associated risks as rupture of an overly distended bladder, peritonitis, laceration of the bladder wall or perforating the wrong structure as a vessel or the intestine (Sink and Weinstein, 2012). Because of the perforation trough the abdominal wall and bladder an elevated number of red blood cells and fat droplets might appear in sediment (Reine and Langston, 2005).

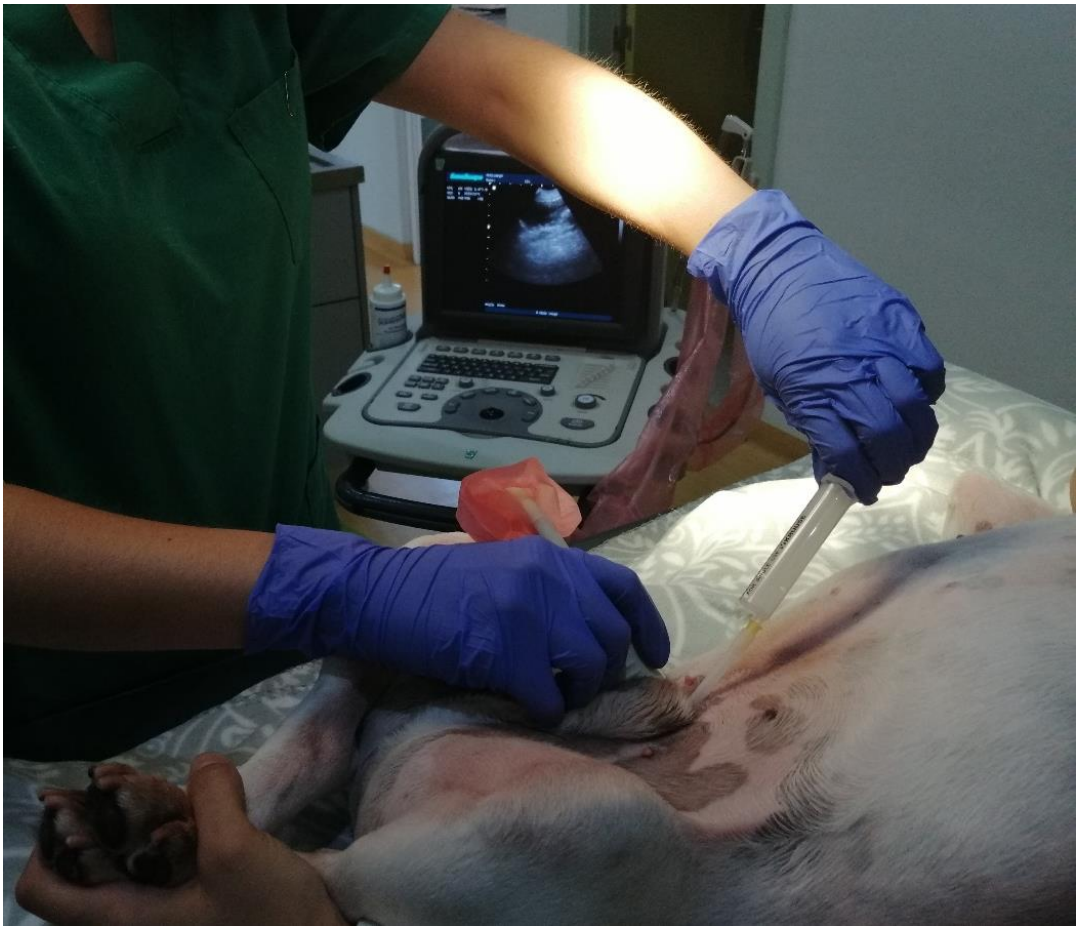

Figure 5 Demonstration on how to perform a cystocentesis using ultrassound. The patient was on dorsal recumbency and held by and assistant. Using ultrassound the urinary bladder was identified, a 10 mL syringe attached to a 21 gauge needle is inserted through the abdominal wall caudally in a 45 degree angle (author image)

3. Complete Urinalysis

The urine sample subdue to urinalysis should be free of contaminants, by collecting the sample to a clean, sterile and hermetic container. However, collecting urine in clean and sterile conditions by the clinician is not always possible, so pre-analytical bias on the collecting method must be considerate when interpreting the complete urinalysis (Callens and Bartges, 2012).

Collecting methods, timing of collection, previous applied therapies and sample management until urinalysis is performed, are all factors that can influence and change the results obtained. The ideal urine submitted to urinalysis should be collected mid-stream by free catching into a sterile container or by cystocentesis. Sometimes it is necessary to collect urine from the worktable or from the floor and so, those samples can be contaminated by disinfectants

or residues, that can influence values of glucose, pH and proteins (Alleman and Wamsley, 2017).

Timing of urine collection, storage time of vesical urine and previous water or food intake should be taken into account when performing urinalysis. Collecting a random urine is preferable, once a urine collected at a determined time of the day can lead to results that in nothing depicts the reality of that patient. As an example, if the collected urine is the first morning urine, the first urine of the day after a long period of being storage in the bladder, can lead to changes in urinalysis such as the bacteriological culture, since it can decrease pathogenic microorganisms viability, and lead to false negative urocultures. In the same way cellular morphology and pH of the urine can be altered by the presence of nitrogen compounds that had been stored during a long period (Cavanaugh and Perazella, 2019).

Urinalysis is an exam that should be made in order to assess a sample representative of a patient's *in vivo* urine, in this manner the sample should be analyzed within minutes after collecting. If necessary, urine samples can be refrigerated for a period of 12h to be analyzed after, being that when they are refrigerated, refrigeration should be done as soon as possible after collection (Alleman and Wamsley, 2017). The laboratory where the urinalyses are performed should be warned that the sample has been refrigerated or should be noticed of the time of urine collection because it can lead to biased results, such as higher crystal formation associated with lower storage temperatures. A prolonged storage time at room temperature will lead to cell degeneration, increased crystals formation and bacterial overgrowth which will then cause secondary parameters changes as pH alterations and decreased glucose values. If exposed to light and air, the sample will suffer photodegradation, oxidation and evaporation (Callens and Bartges, 2012).

Urine examination should start with the evaluation of its physical characteristics, followed by a chemical analysis by urine dipstick. The third step for urine evaluation to be considered complete is the analysis of urine sediment (Ince *et al.*, 2016). Even though, a urine dipstick without abnormal findings in healthy patients can indicate that it is not necessary to proceed to urine sediment analysis it is a test with low sensitivity and negative predictive value, thus, further analysis is recommended when a genitourinary pathology is suspected (Kouri *et al.*, 2000).

3.1. Physical Characteristics

Urinalysis starts at distance, by observing, in a subjective way, physical characteristics that are described according to the observer, and building up to a more profound analysis step by step. Are reported as physical characteristics of urine the color, appearance, the presence or absence of sediment, odor, pH and urinary specific gravity (USG).

3.1.1. Color

Normal urine color varies from light yellow to amber. Pre-analytical factors can affect urine color, such as diet, drug administration and hydration status. A dehydrated patient produces less quantity and more concentrated urine, thus darker, unlike a polyuric animal which produces much more urine and almost transparent. Pigments as hemoglobin, myoglobin or bilirubin are the most commonly found in urine that confer different colors and shades (Bartges, 2009). A red to brown-red tonality is usually related to presence of erythrocytes, hemoglobin or myoglobin in urine, all pigments that, as well as bilirubin that confers a brown-greenish or yellow-greenish tone, are responsible for most pigmenturia. A green color can be described in urinalysis and is, most of the times, related to lower urinary tract infections by pseudomonas, can also be associated with bilirubin oxidation to biliverdin or administration of methylene blue. When methemoglobin forms from hemoglobin in acidic urines a dark brown to black color can appear. Porphyrins and food coloring can also be responsible to different colors appearing in urine, although these are very rare situations (Chew, Dibartola and Schenck, 2011).

3.1.2. Appearance

Urine appearance is usually defined in a subjective scale from clear to flocculate. Normal urine should be clear and when it isn't, urine has to be submitted to a microscopic examination to determine the causes of modified appearance (Piech and Wycislo, 2019). Classifying urine as cloudy is mostly due to an increased number of cellular elements such as leukocytes, erythrocytes, epithelial cells, crystals, mucous and casts. Other elements such as bacteria, fungi, sperm and prostatic secretions also contribute to an increased turbidity of urine.

Leukocytes aggregates, high number of epithelial cells, small calculi and crystal aggregates are responsible for a flocculate aspect of urine (Reine and Langston, 2005).

3.1.3. Odor

Normal urine odor is described as *sui generis*, which is due to the volatile fatty acids present in urine. An ammonium smell can be identified when urease producing bacteria are present, while a rotten smell can be scented in samples that were stored for a long period due to protein putrefaction (Chew *et al.*, 2011).

3.1.4. Urine Specific Gravity

The urinary specific gravity measured by refractometry takes into account the quantity and weigh of molecules dissolved in urine and compares the weight of the urine with the weight of distilled water. This information reflects the capacity of renal tubules to reabsorb water and produce a concentrated urine. USG is directly related to the number of molecules dissolved in urine; however, the value can be overestimated if cells, crystals, mucus or bacteria are present (Wolf, Pillay, 1969; George, 2001). Thus, to try to overcome this overestimate of the USG by refractometry it should be evaluated using the supernatant after the urine is centrifuged (Alleman and Wamsley, 2017). It is described in literature that urine's temperature influences USG once, most fluids at lower temperatures are usually denser than fluids at higher temperatures, but a study performed by Hasan Albasan and collaborators demonstrates that, in their study, the USG is not significantly affected by temperature, being the mean value variation 0.0001 +/- 0.0003 from baseline (Albasan *et al.*, 2003). USG should be interpreted considering multiple factors such as the animals' hydration status, electrolytic concentration, serum creatinine and urea nitrogen, previous administration of certain drugs (corticosteroids, diuretics, antiepileptics) and fluid therapy. Also, some patients' intrinsic pathologic situations, as diabetes mellitus, can cause polyuria / polydipsia which will dissemble the kidneys capacity to concentrate urine. For the multiple causes that can alter cats and dogs USG, reference intervals were determined to be considered as "normal": Dogs USG are considered in the normal range when the values vary from 1.015 to 1.045 and in cats from 1.035 to 1.060 (Rizzi *et al.*, 2017).

Cats urine is naturally more refractive than human or dogs' urines. Thus, cats urinary USG can be overestimate. To overcome this issue specific veterinary refractometers can be used once these differ cat from dogs' urines. If the refractometer doesn't differ different species urines, a formula can be used to deduce feline USG when a human refractometer is used (Alleman and Wamsley, 2017):

$$\text{Feline USG} = (0.846 \times \text{human USG}) + 0.154$$

Refractometers (**Figure 6)** have other limitations, such as the fact that most of manual refractometers have a scale to a maximum of 1040, and it might be needed to dilute the sample using an equal volume of water to know the exact value. There are already digital refractometers capable of reading values until 1080 although a study by Harold W. Tvedten and Asa Norén showed that digital refractometers have a tendency to show lower values when compared to the values obtained with the manual refractometer (Goldberg type refractometer). This way, the values obtained have to be interpreted by the clinician with the clinical history, clinical signs and complete urinalysis (Tvedten and Norén, 2013). Inaccurate USG values can induce the clinician to wrongly undiagnosed renal or endocrine pathologies (Piech and Wycislo, 2019).

Figure 6 A manual refractometer used to measure urine specific gravity. A drop of urine is placed in the refractometer. The refractometer should be pointed to light so that the values can be read in the correct scale identified as urine S.G. (author image).

3.2. Chemical Analysis

The urine chemistry analysis is performed using one of the variable available chemistry urine strips in the market. The urine strips are impregnated with colorimetric reagents that react in the presence of urine, more specifically reacting to the substances present, changing the color or it's intensity according and proportional to the concentration of the substance being measured (**Figure 7**). Color changes might be slight and visual acuity for color determination vary from one individual to another, leading to variations in the interpretation of the test strip results. Multiple urine test strips are available in the market, both human urine test strips and veterinary specific urine test strips that do not account for USG, urobilinogen, nitrite and leukocyte esterase (values not reliable for veterinary patients) (Rizzi *et al*., 2017).

Automatic dipstick scans (**Figure 8**) were developed in order to reduce the human error reading the colorimetric changes, and a study was developed in that order which demonstrated a good correlation for most measurements comparing the automated analysis with the reference methods, except for the values of USG and leukocytes (which presented low sensitivity). This study showed a clear advantage in using the automated analyzer since it reduces the total turn-around time for urine analysis, excludes the human manual data transcription errors and standardizes the procedure reducing the over/underestimation of less experienced operators as previous studies shown it happened (Bauer *et al.,* 2008).

Figure 7 Automatic urine dispstick scan PocketChem UA and specific urine dipstick for automated analysis. The automatic scan is interpretaded and the results are printed automatically (author image).

Figure 8 Urine test strips available on the market. These color strips are dipped in urine. After a minute color change should be interperted according to scale provided by the manufacturer (author image).

3.2.1. pH

Urine pH evidences the ability of the kidneys to conserve hydrogen ions, providing a rough estimate of the body's acid-base status. In cats and dogs the normal urine pH is in a range of 6.0 to 7.5 although changes in it cannot be used as certain estimate of the body's pH since it's highly influenced by diet, recent feeding, bacterial infection, storage time, metabolic and respiratory alkalosis and urinary retention (Parrah *et al*., 2013). The pH of the urine can be determined using urine dipsticks, which gives a semi-quantitative value that might not be accurate due to possible contamination of the reagent of the adjacent pad or because the dipsticks are not veterinary specific, situations to which pH-meters can be presented as a solution (Athanasiou *et al*., 2018).

Urines pH values can be affected by intrinsic or post-collection factors. An alkalization of urine occurs in the postprandial period due to the buffering that occurs in response to the gastric acid release (Parrah *et al*., 2013), when urine is retained for a prolonged time (such as overnight urine) and renal acidosis (Alleman and Wamsley, 2017). Delays in measuring the urine pH may overestimate this value since right after collection the carbon dioxide will diffuse trough the sample over time.

An over acidic urine or the impossibility to alkalinize urine might be associated to various pathological situation such as a urinary tract infection, situations of increased protein catabolism (fever, anorexia), hypokalemia or metabolic acidosis. Severe diarrhea, metabolic alkalosis with paradoxical aciduria associated with gastrointestinal disorders (severe and acute vomiting or gastrointestinal obstruction), Fanconi syndrome and acidifying therapy are other causes of urine acidification (Alleman and Wamsley, 2017).

Some pH results might be falsely increased or lowered due, mostly, to human errors during the handling and the testing of the sample. For example, the contamination of the pH test pad by the acid buffer from protein test pad shows a more acidic urine, or the contamination of the sample with cleanser residues or delays in urine pH measurements will increase the pH values (Rizzi *et al*., 2017). A discolored urine can also lead to a misinterpretation of the results (Alleman and Wamsley, 2017).

3.2.2. Proteins

Healthy dogs and cats without nephropathies usually present a small amount of urine protein, but when an abnormal excess of protein (as albumin, globulins and others) is present the animal is considered to have proteinuria (Lees *et al.*, 2005; Gizzarelli *et al.*, 2019; Vilhena *et al.*, 2015). Proteinuria must be carefully investigated since it has been showed to be not only a marker of nephropathy but also a prognostic factor (Harley and Langston, 2012; Jacob *et al.*, 2005).

False negative results are rare but can happen when a low-level albuminuria (microalbuminuria) or a proteinuria with origin in other proteins than albumin (hemoglobin, immunoglobulins) is present. On the other hand, false positive results are a more common situation that can be cause by an improper storage of the dipsticks (when exposed to humidity), in alkaline or extremely concentrated urines, in contaminated samples with cleanser residues (chlorhexidine and quaternary ammonium compounds), when elements like casts, cells and microorganisms are present, administration of phenazopyridine and, in cats, from high level of cauxin (Alleman and Wamsley, 2017).

3.2.3. Glucose

Glucosuria occurs when the renal threshold for tubular reabsorption is exceeded, being of 180-220 mg/dL (milligram per deciliter) in dogs and 200-280 mg/dL in cats. Diseases as diabetes mellitus, hyperadrenocorticism, acromegaly and pheochromocytoma are conditions that cause persistent serum hyperglycemia leading to glucosuria. Disorders that cause proximal renal tubular dysfunction (Fanconi syndrome, primary renal glycosuria) or renal tubular damage by infection, hypoxia or drugs (aminoglycosides, amphotericin B) and hypercalcemia result in decreased glucose reabsorption without hyperglycemia. Other situations can cause temporarily glucosuria, such as medication or fluids containing glucose, pancreatitis, stress and postprandial periods (Rizzi *et al*., 2017; Heiene *et al,* 2010).

Although most literature refers that no glucose is present in urine samples in healthy animals a recent study performed by Zeugswetter and collaborators proposed the implementation of the term "basal glucosuria". This study verified the presence of glucosuria in euglycemic cats that had no underling pathologies such as kidney disease, hyperthyroidism, diabetes mellitus or were under glucocorticoid therapy that could justify the presence of glucose in urine. Thus, maybe it is of interest to apply the term basal glucosuria in cats as it's already done in humans (Zeugswetter *et al.*, 2019).

False-negative results can appear in samples where bacterial overgrowth occurs *in vitro*, which should be confirmed in the sediment. Moderate ketonuria can lower the results of glucosuria, as it happens with some drugs (ascorbic acid, tetracyclines and methenamine). High USG, cold urine and the use of dipsticks exposed to sunlight are also explanations to a low result of glucosuria when a higher value is expected and should lead the veterinary surgeon to further investigate the result. False positive results can happen in cases of contamination of dipsticks or urine with oxidizing chemicals, prolonged exposure of dipsticks to air and treatment with cefalexin (Alleman and Wamsley, 2017).

3.2.4. Ketones

Ketones are normally produced in small quantities when fatty acids are catabolized to produce energy and are not detected when an animal is healthy and has a balanced nutrition. There are conditions that require a higher carbohydrate metabolism to meet the energy requirements such as diabetes mellitus, renal glucosuria, starvation and pregnancy. A compensatory increase in lipid catabolism occurs as an alternative source of energy to help the organism meet the energy demands which will increase the ketone byproducts in blood. This increase is first noted as ketonuria before it develops as ketonemia, once ketones are quickly removed from the circulation by the kidneys (Alleman and Wamsley, 2017).

Results can be affected by improper storage of the dipsticks or the urine samples, contamination of adjacent pads, urinary tract infections, proximal renal tubular disease and drugs as captopril and N-acetilcysteine. (Alleman and Wamsley, 2017).

3.2.5. Blood

Some dipsticks test pads for blood react with heme molecules (Rizzi *et al*., 2017) so they are incapable of differentiate among erythrocytes, hemoglobin released from erythrocytes in urine, hemoglobin released from systemic hemolysis and myoglobin. The dipstick result should be, thus, interpreted with the analysis of the urine sediment (presence or absence of erythrocytes) and the color of blood serum. There are reagent pads able to differentiate pigmenturia due to red blood cells from hemoglobin in urine, appearing as a speckled color reaction, if in the presence of erythrocytes, and as a diffuse color reaction if hemoglobinuria is present (Chew *et al.*, 2011).

It is of extreme importance to interpret hematuria, defined as more than 5 red blood cells per high power field (RBC/HPF) (Forrester, 2004), with the clinical history of the animal, once the history and the urinalysis together can support or indicate a diagnosis. For example, when hematuria is easily identified at the naked eye, the moment of voiding at which this hematuria occurs is extremely important since it can guide the clinician. A false-hematuria can appear in cases where the blood is actually dripping from penis or vulva independently from urination, indicating a probable bleeding distal from the urethral sphincter (prostate, urethra, penis, uterus, vagina or vulva) (Alleman and Wamsley, 2017).

3.2.6. Bilirubin

Billirrubin can physiologically appear as trace to 1+ in dogs urine dipsticks chemical analysis due to the low renal threshold to bilirubin, particularly in concentrated urines. In cats the threshold for bilirubin it's higher, so when bilirubin is present is pathological (Rizzi *et al*., 2017).

Bilirubinuria usually precedes jaundice and bilirubinemia as a clinical sign that can be present in cases of hepatic or post-hepatic cholestatic diseases, hepatic parenchymal disorder (decreased bilirubin uptake or conjugation), hemolytic diseases and prolonged anorexia or fever. False-negative results appear when urine is exposed to light for more than 30 minutes or air, prior centrifugation of urine before testing or the administration of ascorbic acid. Drugs as etodolac metabolites and phenazopyridine can lead to false-positive results (Alleman and Wamsley, 2017).

3.2.7. Urobilinogen

This test has very little significance in veterinary medicine and it is common to appear a small amount (0.2mg/L) in healthy animals. The values might be increased in hemolytic or hepatobiliary disease or decreased due to bile duct obstruction or simply due to diurnal variation (Alleman and Wamsley, 2017).

3.2.8. Others

In urine dipsticks values of nitrites, USG and leucocytes can appear, but these shouldn't be considered since they are unreliable in veterinary medicine (Alleman and Wamsley, 2017).

3.3. Urine Sediment Microscopy

The microscopic examination of urine sediment should not be overlooked since it contains meaningful information, being comparable to performing a differential blood count as part of a complete hemogram. The examination of the urine sediment can help the clinician to perform diagnosis (Chew *et al.*, 2011; Verdesca *et al.*, 2007).

The urine sediment is prepared centrifuging a standard volume of urine (usually 2, 5 or 10 mL) at 1500 to 2000 rpm (rotations per minute) for 5 minutes. The sediment correlates to the number of cells, casts, crystals and other materials that might be present in urine that concentrates at the bottom of the tube. When the quantity of elements is high the sediment might be visible at the naked eye.

After centrifuging the supernatant is removed by aspiration or simply turning the tube upside down and letting the supernatant drop. Only two or three drops of urine are needed to resuspend the sediment which can be done with the help of a pipette, flicking or shaking the tube; either technic of resuspension should be done gently not to cause cellular artifacts or disrupt casts (Rizzi *et al.*, 2017).

To evaluate the urinary sediment a drop or two of the re-suspended sediment are placed in a clean glass slide and covered with a coverslip to be visualized under microscopy (**Figure 9**). For manual microscopic observation of urine phase contrast microscopy is recommended, even though CLSI guidelines state that bright field microscopy is enough to evaluate and correctly diagnose genitourinary pathologies (Fogazzi and Delanghe, 2018; Kouri *et al.*, 2000; Rabinovitch *et al.*, 2009).

The sediment can be observed either with or without staining. Staining can be used to help identify some elements or particles, mostly bacterial agents. It should be considered that urine sediment stain introduces variations such as dilution, contamination, stain precipitation and the staining quality is variable. Thus, the urine sediment should be observed before and after the staining (Brown, 2011).

The unstained urine sediment is examined under the light with the condenser lowered, which provides the contrast to identify the elements present in the sediment. If the sediment is stained it should be examined with the condenser raised and with increased light (Chandrashekar, 2018). The sediment is examined under low power field (10x objective) to evaluate the general composition and focusing of structures. The observation on high power field (40x objective) is used to determine sediment content, with observation of at least 10 fields. The number of elements described in the report correspond to the mean value of the total fields observed (Sink and Weinstein, 2012; Callens and Bartges, 2012).

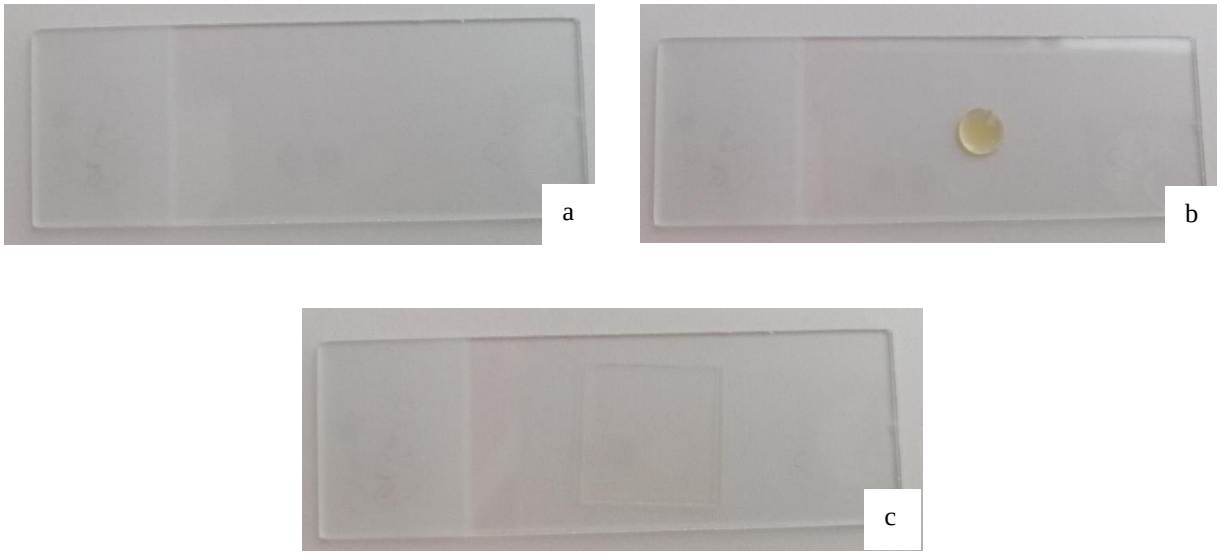

Figure 9 Representation on how to prepare the traditional method of urine sediment. (a) a clean microscopic slide is used where (b) a drop of centrifuged urine is placed on the microspic slide and (c) covered with a coverslip to be observed under ligh microscopy (author image)

3.3.1. Red blood cells

A reduced number of erythrocytes can be present in urine sediment of healthy dogs and cats, and the number of red blood cells accepted varies according to the collection technique (voided, manual compression, cystocentesis) (Forrester, 2004), but generally a mean of 5 RBC per HPF is considered the upper limit of reference range for healthy animals (Rizzi *et al.*, 2017).

Red blood cells, in cats and dogs, are biconcave disks, without nuclei and, in unstained sediment, have a pale color. They should be microscopically distinguished from similarly shaped elements including yeast, lipid droplets and air bubbles. Erythrocytes are usually all the same size, unlike other elements, and smaller than leukocytes, showing a smooth appearance (**Figure 10**). If needed acetic acid can be added and RBC will suffer lyse while yeast and lipid droplets will remain intact (Sink and Weinstein, 2012).

The normal morphology of erythrocytes can vary according to the osmolality of the urine. In concentrated urine the RBC will lose water and appear crenated, being pale, spherical and with irregular surface projections, that can refract the light, giving the erythrocytes a ‘’glitter’’ appearance. In hypotonic urine erythrocytes swell and may suffer lysis. Before lysis occurs, the erythrocytes appear larger and rounded rather than biconcave. Lysed erythrocytes may appear faint (ghost cells) or not visible (Rizzi *et al*., 2017).

3.3.2. White Blood Cells

White blood cells are important to differentiate from other elements, so the clinician is able to define leucocyturia. Leucocytes are approximately 1.5 times larger than RBCs and have nucleus (**Figure 11**) (Sink and Feldman, 2004). Neutrophils constitute most of the white blood cells population found in urine sediment, appearing as spherical, colorless with grainy cytoplasm cells, where, sometimes, the lobulated shape of the nucleus can be identified (Rizzi *et al.*, 2017). Other WBCs can be seen in urinary sediment, although in less quantity, such as eosinophils, which are similar to neutrophils, that can be easily distinguished if stained, lymphocytes, which have a higher nuclear to cytoplasm ratio, monocytes and macrophages, that are uncommon and usually larger, with a easily identified ovoid to lobulated nucleus and abundant cytoplasm (Sink and Weinstein, 2012).

A small amount of white blood cells is considered normal in urine sediment and the minimal number of cells accepted might depend on the collecting of the sample. According to literature is considered normal to find up to 8 WBCs per HPF in voided samples, up to 5 WBCs per HPF in catheterized samples and up to 3 white blood cells in samples obtained by cystocentesis (Chew *et al.*, 2011).

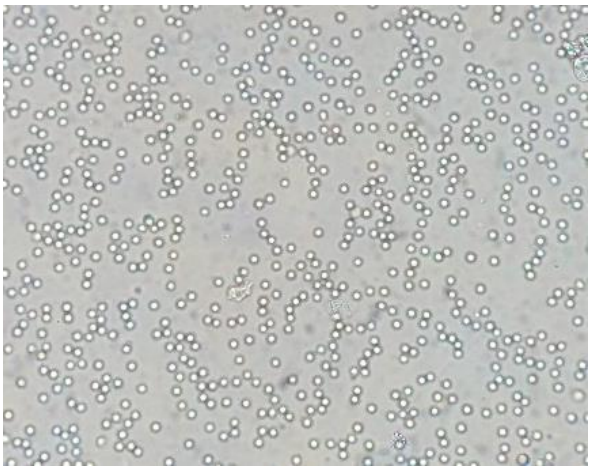

Figure 110 A microscopic field of a hematuric urine. Multiple red blood cells appear as spherical pale cells without nuclei (author image)

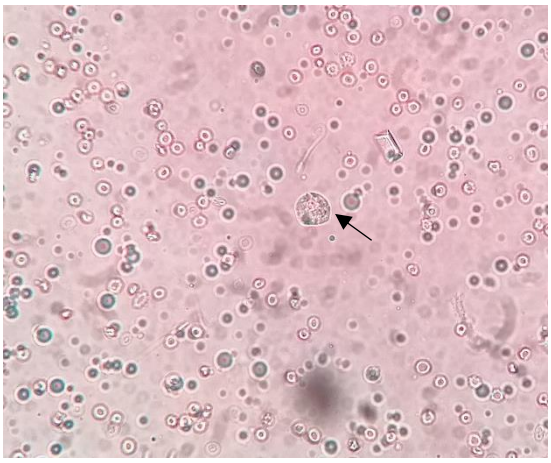

Figure 101 Presence of a white blood cell (arrow) in a field of a urine with hematuria, crystalluria and various contaminants. White blood cells appear as round colorless cell with preeminent grainy cytoplasm (author image)

3.3.3. Epithelial Cells

Due to the normal cellular turnover of the genitourinary tract it is normal to find some epithelial cells in a routine urinalysis (up to five cells per low power field (LPF)). In wet mount preparations it can be difficult to distinguish different types of epithelial cells once their all highly pleomorphic and they all become round due to the surrounding environment and because of the prolonged exposure to urine cells degenerate (Batamuzi and Kristensen, 1995; Alleman and Wamsley, 2017).

3.3.3.1. Transitional epithelial cells

Transitional epithelial cells can vary in size but are normally medium-sized, round, oval or polygonal cells with a large round to oval central nucleus, larger than squamous epithelial cells, and with granular cytoplasm (**Figure 12**) (Batamuzi and Kristensen, 1995). Transitional epithelial cells can appear individually or in groups but should be less than five cells per low power field. These cells arise from renal pelvis, ureters, bladder and proximal two thirds of urethra, and are present in a larger number if the sample is collected by catheterization or in urinary tract pathologies such as inflammation, hyperplasic or neoplastic mucosa (Rizzi *et al.*, 2017).

3.3.3.2. Squamous epithelial cells

Squamous cells arise from the distal third of the urethra, vagina and prepuce. They appear as large flat or rolled cells, with angular sides and usually a single small condensed nucleus (they may appear anucleate) (**Figure 13**). The number of squamous cells present in urine sediment depend on the collecting method, if the urine is collected by cystocentesis no squamous cells should be present, whilst in voided or catheterized samples is normal to see a variable number of cells. In rare occasions squamous cell can be identified in urine sediment of samples collected by cystocentesis in patients with squamous cell carcinoma of the bladder or transitional cell carcinoma or chronic bladder irritation (Alleman and Wamsley, 2017).

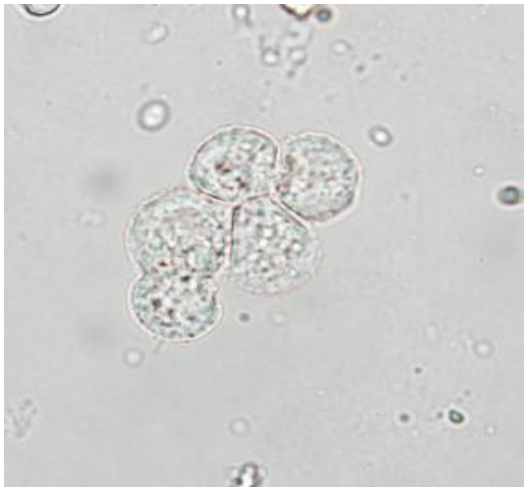

Figure 12 Transitional epithelial cells appear as round large cells with round nucleus and granular cytoplasm (adapted from Rizzi *et al.*, 2017).

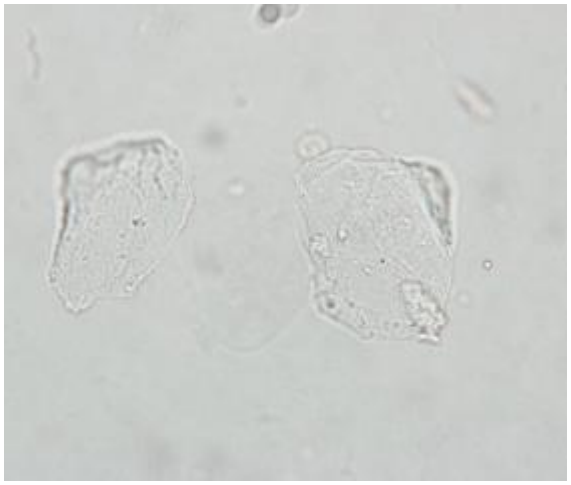

Figure 13 Squamous epithelial cell are large flat cells with angular sides and a small nucleus (adapted from Rizzi *et al.*, 2017)

3.3.3.3. Renal tubular epithelial cells

Renal tubular epithelial cells are rarely seen in urinary sediment, mostly due to their degeneration as a result from the transit time from renal tubules. These cells appearance varies according to the area of renal tubules from which they originate but are mostly small and cuboidal (Sink and Weinstein, 2012). An abnormal number of renal tubular epithelial cells can be an indicator of renal tubular disease (Chawla *et al.*, 2008; Kanbay, Kasapoglu and Perazella, 2010), although this situation should be explored once these cells can be mistakenly identified when leukocytes or small transitional epithelial cells are present (Alleman and Wamsley, 2017).

3.3.3.4. Neoplastic epithelial cells

In urinary sediment of a patient with a bladder or urethral mass in which atypical transitional epithelial cells appear without signs of inflammation the clinician should suspect of transitional cell carcinoma. These cells can appear individually or in a form of cellular casts and their morphology has the malignant characteristics of other neoplastic cells: higher nuclear to cytoplasmic ratio, variable size between nuclei, aggregated chromatin with prominent nucleoli and several mitotic figures. When inflammation is present concomitant with atypical transitional epithelial cells the diagnosis of transitional cell carcinoma can't be made due to its difficulty to distinguish from hyperplasic epithelial cells. For definitive diagnosis other diagnostic exams should be performed, such as histological biopsy and imprint cytology, since urinary cytology is not a definitive diagnostic test (Alleman and Wamsley, 2017).

3.3.4. Casts

Several types of casts can be identified in urine sediment according to which cells (if any) or cellular debris incorporates the core of the cast (it may be of only one type or a mixed cast). Tamm-Horsfall mucoprotein (THM), secreted by the cells of the ascending loop of Henle, distal tubules and collecting ducts, forms the matrix of all casts. Casts appearance depend on the length and widening of the tubules where they're formed, for example, if formed in the ascending limb of the loop of Henle casts are longer than they are wide, whether if they're formed in the collecting ducts as they have large lumens casts will appear larger. Factors as decreased flow rate and urine pH, high solute concentration and increased proteins promote the secretion of THM and casts formation, leading to cylinduria when the number of casts in urinary sediment is increased (Serafini-Cessi *et al.*, 2003; Devuyst *et al.*, 2005). Cylindruria can happen with diseases affecting any area of the kidney (Chawla *et al.*, 2008).

Pseudo casts appear to be casts at first glance but lack of some cast characteristics as parallel sides and other features of specific casts (Alleman and Wamsley, 2017).

3.3.4.1. Hyaline Casts

Hyaline casts appear semitransparent in unstained samples once they have a low refractive index (similar to that of urine), are pale and colorless. Usually one of the ends of hyaline casts is blunt and round while the opposite end resembles a tail. To identify these casts is mandatory that the microscopic condenser is lowered, and a careful identification has to be made because they can be mixed with granular casts because of amorphous debris that frequently adheres, in an uneven distribution, to the outside surface of these casts (Rizzi *et al*., 2017).

These casts are composed of THM without any cells or cellular elements. Up to 2 casts per LPF are considered normal in concentrated urines of both dog and cat, yet a higher number of hyaline casts found can both represent a physiologic or pathological condition. Fever, strenuous exercise, diuretics and passive congestion are situations in which is physiologic that a patient presents an increased number of hyaline casts. In protein-losing glomerular disease hyaline casts serve as indication for further exploration by the clinician (Caleffi and Lippi, 2015).

3.3.4.2. Cellular casts

Leukocytes, erythrocytes and renal tubular cells can compose the cellular casts when they are incorporated in the THM matrix, whether forming a cast of a single cellular type or forming a mixed cast (when more than one cell type is present). In healthy dogs and cats cellular casts are usually not present and, even in patients with renal disease, they are rarely identified. If identified, these types of casts are related to acute renal injury. If only one cellular type constitutes the cast, it can be identified according to those cells (Rizzi *et al*., 2017).

Epithelial cell casts are composed of intact, peeled renal tubular epithelial cells, which can mistakenly be identified as granular casts when casts are not shed into urine for a prolonged time due to cellular degeneration. The presence of epithelial cell casts indicates an active renal tubular necrosis or acute damage, suggesting a severe intrarenal disease. Infarcts, ischemia, acute nephritis and renal toxins are associated with acute renal damaged, hence with epithelial cellular casts (Fogazzi *et al.*, 2012).

Cellular casts where leukocytes are the primarily cellular population of the casts are described as white cell casts (although it can include renal tubular cells or fragments of these). Neutrophils are the most common leukocyte caught up in the THM matrix, for their segmented nucleus, when cellular detail is good, is easy to identify these casts. The observer should not confuse clumps of leukocytes with white cell casts. Septic or non-septic intrarenal inflammation can lead to white blood cell casts, most commonly acute bacterial pyelonephritis, even though interstitial nephritis can also be pointed as a cause (Praga and González, 2012).

As white blood cell casts, also red blood cell casts are formed which acquire an orange to yellow appearance when included in the THM matrix. Red cells casts are rarely seen because RBCs are fragile and easily dissociate, causing casts to dissociate. When seen in urinary sediment they are always associated with pathologic situations indicating intrarenal hemorrhage due to renal trauma, hemorrhagic nephritis or glomerulonephritis (Fogazzi *et al.*, 2012).

3.3.4.3. Granular casts

Granular casts contain particular matter (cell fragments from renal tubular cell necrosis or degeneration) with different sizes and shapes, which will lead to more fine or coarse casts. Depending on the density, size and granulometry they may appear gray to yellow to black. Granular casts are longer than larger, with irregular sides, but due to their fragility they can appear with broken ends, hence, shorter. In healthy dogs and cats one granular cast per low power field can be found. If more than one is found is considered a pathological increase, even in concentrated urine (Rizzi *et al.,* 2017). Are causes of granular casts: renal tubular damages, that cause lyse of tubular cells leading fragments to be released and incorporated in the cast matrix; epithelial cellular casts, that suffered degeneration due to a prolonged stay in the urinary tract; in some cases of glomerular disease the protein lost can be incorporated in the casts and give them a granular appearance instead of forming hyaline casts (Caleffi and Lippi, 2015).

3.3.4.4. Waxy casts

Waxy casts appear homogeneously colorless to gray to light yellow, have a higher refractive index being easier to identify than hyaline casts (Caleffi and Lippi, 2015). These casts, like other, have parallel sides and blunt ends, due to their fragility the ends might appear fractured. Waxy casts are not commonly found in cats or dogs and are thought to represent the end stage of granular cast degeneration. They may appear when cellular elements are caught in the THM matrix and are retained in renal tubules or ducts for a long period after acute renal injury or at later stages of a chronic active renal injury. Their presence implies a previous damaged with a long-term nephron obstruction (intrarenal stasis). As in other casts their conformation represents the local where they were formed, so when broad waxy casts are found this indicates that they were formed in the collecting ducts or dilated portions of the distal tubules. Usually the rate flow is higher in this nephrons portions, but waxy casts require stasis of this portions to be formed, which indicates severe renal disease. In the resolution phase of severe renal disease, a high number of waxy casts can appear due to the diuresis phase that the kidney converts to after a period of oliguria (Rizzi *et al*., 2017).

3.3.4.5. Lipid Casts

Also named fatty casts, are granular casts that incorporated a variable number of fat droplets. The fat droplets vary in number between casts and can vary in sized within the same cast, appearing refractile and clear to yellowish. The fat from these casts arise from the fatty renal tubular cells of some cats and diabetic dogs. When the renal tubular cells and cell fragments are incorporated in the THM matrix so is the fat, thus these lipid casts should be interpreted as granular casts (Rizzi *et al.*, 2017).

3.3.4.6. Hemoglobin casts

Hemoglobin casts have a granular texture and yellow to golden-brown color. These casts are most commonly associated to intravascular hemolysis, less often result of renal hemorrhage and subsequent break-down of the red blood cells (Rizzi *et al*., 2017).

3.3.4.7. Bacterial Casts

Bacterial casts might be confused with granular casts, because when they are filled with bacteria the appearance is similar. This finding is rare and suggests a severe renal tubular infection. To a definitive diagnose between granular and bacterial casts a Diff-Quick staining can be made (Perazella and Fogazzi, 2010).

3.3.5. Crystals

The presence of crystals in urine is defined as crystalluria. Crystalluria can appear when urine with dissolved minerals is saturated or due to crystallogenic substances (Fogazzi GB, 1996; Okafor *et al.*, 2019). It is important to notice that the observed crystals under microscopy might be formed *in vivo*, due to pathological or non-pathological reasons, or *ex vivo* due to sample handling (prolonged storage and cold temperatures) (Alleman and Wamsley, 2017). *Hasan* and collaborators concluded, in their study, that 28% of the samples were misinterpreted due to failure to differentiate *in vivo* from *in vitro* crystalluria in refrigerated samples for 24 hours (Albasan *et al.*, 2003).

Most crystals possess characteristic features as shapes and color, that allow identification, however urinary pH can help, since certain types of crystals are more easily formed in a more acidic and other in a more neutral or basic environment (Sink and Weinstein, 2012).

3.3.5.1. Struvite

Struvite crystals, also known as triple phosphate crystals, appear as colorless, refractile prisms that remind a "coffin lid" shape (**Figure 14**). These crystals are formed by magnesium, ammonium and calcium phosphate, thus the triple phosphate crystals denomination. Struvite crystals are the most commonly found in healthy dogs and cats' urine, and therefore struvite uroliths have the higher incidence. Although struvite crystals might be found in healthy animals if persistently present in abundant quantities is an abnormal situation, and to prevent later urolith formation, predisposing causes should be investigated such as bacterial infection of the urinary tract, alkaline urine, increased levels of dietary magnesium and decreased volume of

produced urine. It is reported to be the most frequently found crystal in urethral plugs of male domestic cats (Okafor *et al.*, 2019, Osborne *et al.*, 2009).

Medical management of struvite uroliths is possible and should be tried before surgical removal. Once this type of crystals form in alkaline urine it is important to work through prevention and dissolution of the existing crystals and uroliths by dietary management that will reflect in urines' pH. Besides increasing water consumption to dissolve the existing crystals and providing an acidifying diet balancing acidifiers (methionine, calcium, sodium sulfate and ammonium chloride) and alkanizers to achieve the desired acidification potential and the administration of small meals through the day to the animal will allow to mitigate the postprandial alkaline tide (Queau, 2019).

3.3.5.2. Calcium Oxalate

Calcium oxalate dihydrate crystals appear as colorless, square to rectangular octahedral, with an "X" forming by the connection of the four corners, reminding the aerial viewing of a pyramid (**Figure 15**). Their presentation in size can vary from a very small form that difficult their identification to very large crystals. It can be found a small number of this type of crystals in urines from healthy animals, but when a moderate to abundant number of crystals are present it suggests a pathological situation that should be identified (Rizzi *et al*., 2017). Ethylene glycol ingestion (non-pathognomonic sign), hypercalcemia, hypercalciuria, increased dietary oxalates, found in some plants as beets, beans, potatoes and leafy vegetables and acidic urine can be the cause of this finding (Queau, 2019; Osborne *et al.*, 2009).

Medical resolution of calcium oxalate dihydrate crystals is not possible, so medical management should be applied to animals at risk to have urolithiasis or the ones who've already underwent surgical resolution to prevent recurrences. Increased water intake, either by providing food with higher water content or higher sodium content, will decrease the calcium oxalate RSS (Bartges, 2016). Even though a higher sodium intake could be controversial once it could lead to a higher calcium excretion the dilution achieved will allow urine calcium concentration not to be altered or to even decrease. Diets with higher sodium, moisture, protein and potassium content are associated with a lower risk of calcium oxalate formation. The urinary pH is not of main importance in formation of these type of crystals once, even though

they are more easily formed in acidic urines, they can form in any pH, so it is not important to alkalize urine once it will predispose to formation of struvite crystals (Queau, 2019).

Calcium oxalate monohydrate crystals have the same predisposing causes to appear in urine sediment and are equally treated and prevented such as calcium oxalate dihydrate uroliths when formed (**Figure 16**). Yet calcium oxalate monohydrate crystals are not usually found in healthy animals and have a very different appearance. These crystals appear as elongated, flat, translucent crystals that have both ends pointy, or they might appear completely different as oval (hemp seed), barrel or dumbbell shaped (Rizzi *et al.*, 2017).

3.3.5.3. Calcium Phosphate

Calcium phosphate crystals appear in animals with hypercalciuria or hyperphosphaturia, presenting as colorless plates (thin and flat) or needles (thin and long) (**Figure 17**). Calcium phosphate are rare in dogs or cats. Conditions as hyperparathyroidism, hyperadrenocorticism and distal tubular renal acidosis are predisposing causes for hypercalciuria and hyperphosphaturia (Rizzi *et al.*, 2017). Calcium phosphate crystals form more easily in alkaline urine, thus, diet, even though is not going to resolve the existing crystals or possible uroliths, can be used in management of animals at risk of developing calcium phosphate crystalluria. The management diet should promote a moderately acidic urine pH, promote urine dilution and contain controlled amounts of calcium, phosphorus and vitamin D. The main treatment for calcium phosphate crystals or uroliths is to identify and treat the subjacent pathology (Queau, 2019).

3.3.5.4. Ammonium Urate

Ammonium urate crystals usually appear as yellow brown to brown spheres with long, irregular protrusions, although the spheres can appear with a smooth surface instead with internal radiating striations (**Figure 18**) (Rizzi *et al.*, 2017). These crystals are rarely found in dogs and cats, except in Dalmatians and English Bulldogs where a small to moderate amount of urate crystals can be found without any clinical signs present. For other dog breeds besides Dalmatians and English bulldog urate crystals can relate to conditions as hepatic dysfunction,

particularly portal vascular anomalies. In cats it can be related to hepatic dysfunction, but it is frequently defined as idiopathic (Bartges and Callens, 2015).

Managing ammonium urate crystals focuses primarily in preventing crystal formation in susceptible dogs and cats. The first step, as for other crystals and uroliths, consists in dilution by promoting the water intake. Urates form from catabolism of purines (endogenous or dietary), thus decreasing dietary purine content will decrease purine metabolite excretion. Low purine diet does not necessarily mean low protein. Purine content varies in different protein sources being higher in organ meats and fish and lower in plants and egg white, so a low purine diet can have normal content in protein if the correct sources are used. A more alkaline urine should also be promoted once ammonium urate crystals are less soluble in acidic urine (Queau, 2019).

3.3.5.5. Xanthine

Xanthine crystals are not reliably distinguishable from ammonium urate or amorphous urate crystals by simple light microscopy since their appearance is usually brown or yellow-brown spherules of variable sizes (Rizzi *et al.*, 2017). Xanthine crystalluria can occur secondary to allopurinol administration in dogs who have a diet high in purines (Queau, 2019) or as a result of an inborn error in purine metabolism described in Cavalier King Charles and Dachshund. In cats, spontaneous xanthine crystalluria and uroliths have been described as a probable genetic or congenital defect in xanthine oxidase activity (Rizzi *et al.*, 2017).

Preventing the recurrence or appearance of xanthine crystals and uroliths is based in adjusting allopurinol dosage in patients under such therapy and decrease the purine content of diet. As for other crystals the increase in water intake is of the most importance to promote dilution (Queau, 2019).

3.3.5.6. Uric Acid

Uric acid crystals appear colorless (Alleman and Wamsley, 2017) to yellow or yellow-brownish (Rizzi *et al.*, 2017), flat and usually in a six-sided diamond shape with variable sizes (**Figure 19**). These types of crystals are formed when uric acid is excreted in urine in its native form which happens in dogs that have a diminished hepatocellular uptake for uric acid as

Dalmatians. In normal dogs, uric acid is converted into allantoin, a water-soluble compound, in the liver for excretion. In Dalmatians not only the hepatocellular intake is lesser, but the tubular reabsorption is decreased when compared with other breeds explaining the elevated risk of this breed to develop uric acid crystals and uroliths, on the contrary these crystals are not usually found in cats or other dog breeds, with the exception of English Bulldogs where, as ammonium urate crystals, also uric acid crystals can be occasionally found (Alleman and Wamsley, 2017).

Managing uric acid follows the same steps as managing ammonium urate crystalluria (Queau, 2019).

3.3.5.7. Cystine

Cystine crystal are always an abnormal finding in urine sediment representing a pathology that affects cystine metabolism (Rizzi *et al.*, 2017) due to a genetic defect in proximal renal tubular transport of amino acids (Mizukami *et al*, 2015; Brons *et al.*, 2013). Male Dachshunds, Basset Hounds, English Bulldogs, Yorkshire Terriers, Irish Terriers, Chihuahuas, Mastiffs, Rottweilers and Newfoundlands are more predisposed to cystinuria. These crystals have a flat, colorless and hexagonal appearance with sides that can have equal length or not (**Figure 20**). Cystinuric patients don't always develop cystine crystals and uroliths, although in concentrated and acidic urine they're more prone to develop (Alleman and Wamsley, 2017).

As for other crystals urine dilution is of the upmost importance for treatment. Urine cystine excretion can vary according to diet protein content, specifically according to the content in methionine and cysteine amino-acids, thus a diet with the minimum requirements content in the previous referred essential amino-acids is better to avoid recurrences or preventing the crystals and uroliths formation in predisposed animals. An alkaline urine should be promoted once cystine crystal formation and solubility is highly dependent of urines' pH. A special attention should be taken to cystinuric dogs and cats, accounting that carnituria and taurine deficiency have been reported when dietary management of these crystals is done. Taurine and carnitine are advised to prevent dilated cardiomyopathy, both having methionine as precursor, and for this the diet should be restricted but balanced (Queau, 2019).

3.3.5.8. Amorphous crystals

The denomination of amorphous crystals includes phosphate, urates, xanthine and silicate crystals that appear as small granular crystals without any distinctive features **(Figure 21**). Urine pH can help to identify the types of amorphous crystals present in a sediment, although an accurate definition of amorphous crystals might be difficult once they can be mistaken for degenerating cells, other crystals that are going through degeneration or even bacterial *cocci* (Rizzi *et al.*, 2017).

Amorphous crystals may lead to crystal and urolith formation, so a careful follow-up should be made, and urine dilution should be promoted by increasing water intake (Rizzi *et al.*, 2017).

3.3.5.9. Bilirubin crystals

Bilirubin crystals appear as needle-like, golden to golden-brow crystals (**Figure 22**). Conjugated bilirubin is freely filtrated trough the renal glomeruli, if a hepatic or post-hepatic biliary disease is present the excreted conjugated bilirubin exceeds the reabsorption kidney capacity, leading to bilirubinuria. If bilirubin crystalluria is due to biliary disease the serum/plasma bilirubin will also be increased, although the presence of an occasional bilirubin crystal in a concentrated urine might appear and should not arise alarm to the clinician (Rizzi *et al*., 2017).

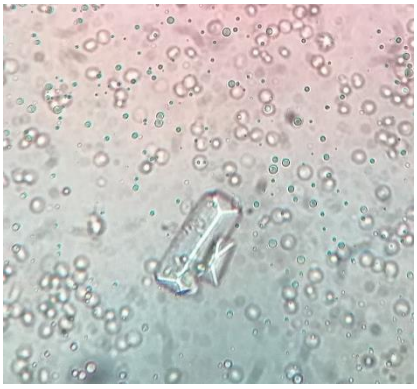

Figure 14 Struvite crystals in a field of hematuric urine (author image)

Figure 15 Calcium oxalate dihidrated crystals (adapted from Rizzi *et al*, 2017).

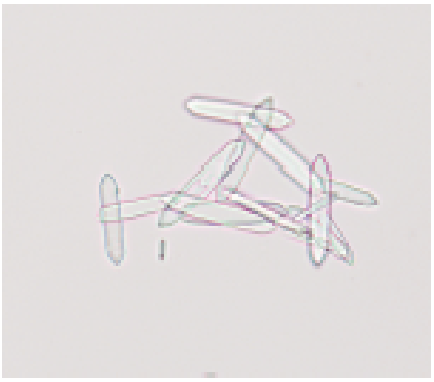

Figure 16 Calcium oxalate monohidrated crystals (adapted from Rizzi *et al.*, 2017).

Figure 17 Calcium phosphate crystals (adapted from Rizzi *et al.*, 2017).

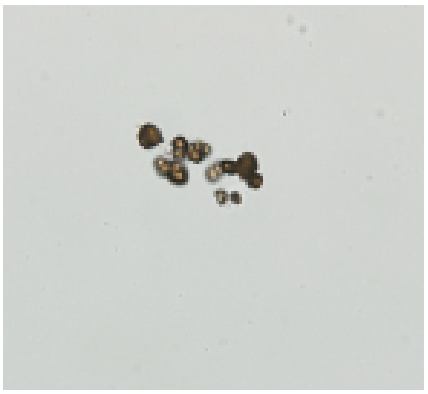

Figure 18 Ammonium urate crystals (adapted from Rizzi *et al.*, 2017).

Figure 19 Uric acid crystals (adapted from Rizzi *et al.*, 2017).

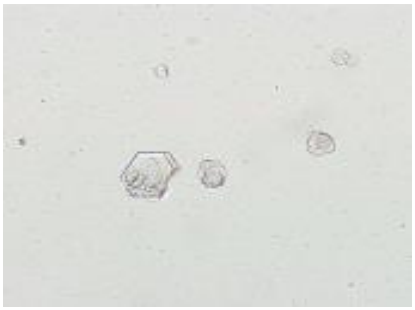

Figure 20 Cystine crystals (adapted from Rizzi *et al*, 2017).

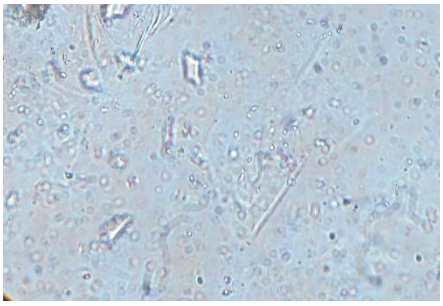

Figure 21 Amorphous crystals in a urine highly contaminated (author image)

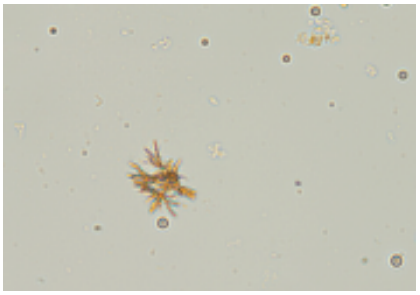

Figure 22 Bilirubin crystals (adapted from Rizzi *et al*, 2017).

3.3.6. Bacteria

Bacterial *cocci* or bacilli can be found in urine sediment. These organisms are usually motile, although *cocci* can be mistaken for amorphous crystals and (Sink and Weinstein, 2012) occasionally they may appear filamentous or in chains and be confused with fungal hyphae. To confirm the presence of bacteria in urine sediment a rapid Romanowski-type stain as Diff-Quick of a dried urine smear can be performed (Rizzi *et al.,* 2017).

In a normal dog or cat the urine in the bladder should be sterile, but in free catch urine and those gathered by catheterization some bacterial contamination might be present, although not in enough number to be seen by microscopy in a fresh urine sample. If these contaminated samples are stored at room temperature, bacteria will multiply and at microscopic examination it might appear as bacteriuria. Usually if bacteriuria is present a corresponding increase of white blood cells (pyuria) appear (Dorsch *et al*, 2019). Sometimes silent urinary tract infections (without pyuria) can happen in animals with hyperadrenocorticism / hypercortisolemia, diabetes mellitus and other immunosuppressor diseases. A special attention should also be taken to patients presenting polyuria of large volumes of urine where the bacteria and leucocytes may be diluted (Alleman and Wamsley, 2017; Byron, 2019).

3.3.7. Yeast and fungi

Yeast appears as colorless, refractile and variable in size, which might be morphological similar to erythrocytes and lipid droplets. When the infection is severe hyphal structures can be observed. When differentiation between red blood cells and yeast is difficult adding acetic acid to the sample will cause the RBCs to lyse leaving yeast intact (Sink and Weinstein, 2012). As an organism from the normal flora of the urogenital tract *Candida spp* is the most frequently found yeast (Pappas *et al.,* 2018) and an increased number indicates an underlying cause as recent antibiotic treatment, glucosuria and other urinary tract diseases (Reagan *et al.,* 2019). *Candida spp* may present in a pseudohyphae form and should be differentiated from fungi, as aspergillosis, that appear in the hyphal form. Fungal infection rarely manifests in urine and, when present, represents a systemic fungal disease with renal involvement (Sink and Weinstein, 2012).

3.3.8. Spermatozoa

Spermatozoa are expected to be seen in urine samples from intact males, even if the sample is obtained from cystocentesis, and can also be identified in females after breeding in urine samples obtained by free catch or catheterization (Brown, 2011). The spermatozoa are easily identified for their oval body with a long flagella-like tale attached to it by a central rectangular mid-piece. They're usually non-motile in urine sediment (Sink and Weinstein, 2012).

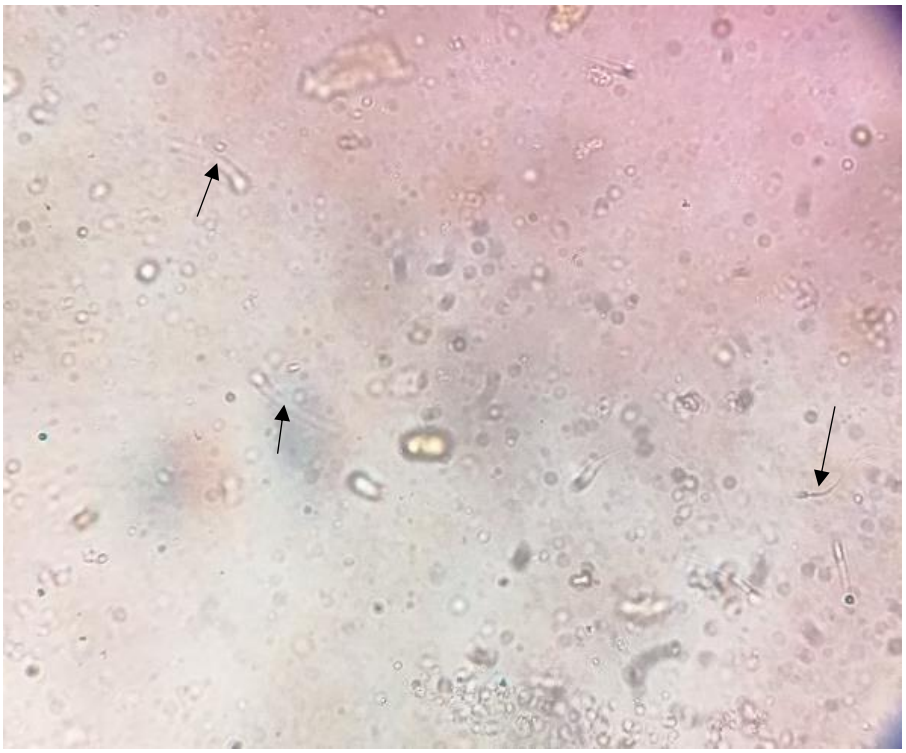

Figure 23 Spermatozoa (arrows) appearing in a male dogs' urine as a contaminant of a free catch sample (author image)

3.3.9. Parasites

The identification of different parasites it's highly dependent on the geography, according to their distribution (Alleman and Wamsley, 2017). It is important to notice that parasite ova identified in urine sediment might result from fecal contamination (Sink and Weinstein, 2012).

In urine sediment nematode ova, larvae or adults of *Capillaria spp, Dirofilaria immitis* microfilarae, *Dioctophyma renale* and *Trichuris spp* can be identified. It can be difficult to differentiate eggs of *Trichuris spp* from eggs of *Capillaria spp* and a careful examination of their bipolar caps and outer shells should be made, once *Trichuris spp* caps are perfectly bipolar and has a smooth appearance of the shell and *Capillaria spp* has slightly askew caps and a shell with a granular appearance (Alleman and Wamsley, 2017).

3.3.10. Mucus

Mucus appears as long, thin structures with low refractive index in unstained urine, if the urine sample is stained the mucus present might difficult the examination of the urine sediment once it stains very dark. Strands of mucus can be mistaken for granular or hyaline casts although they're usually more irregular in shape and have taper ends. When mucus is present in urine of dogs and cats it is frequently associated with inflammation of the urogenital tract (Rizzi *et al*., 2017).

3.3.11. Contaminants and artifacts

It is commonly found in urine samples contaminants as dust particles, hair, starch granules, glass chips, fiber (**Figure 24**) from cotton and wool and pollen (Sink and Weinstein, 2012).Talc and glass shreds can be mistaken for urine crystals, plant pollen as transitional epithelial cells and hair or fibers for casts, thus the importance of collecting urine in the most aseptic way and always indicate how the urine was collected (Alleman and Wamsley, 2017).

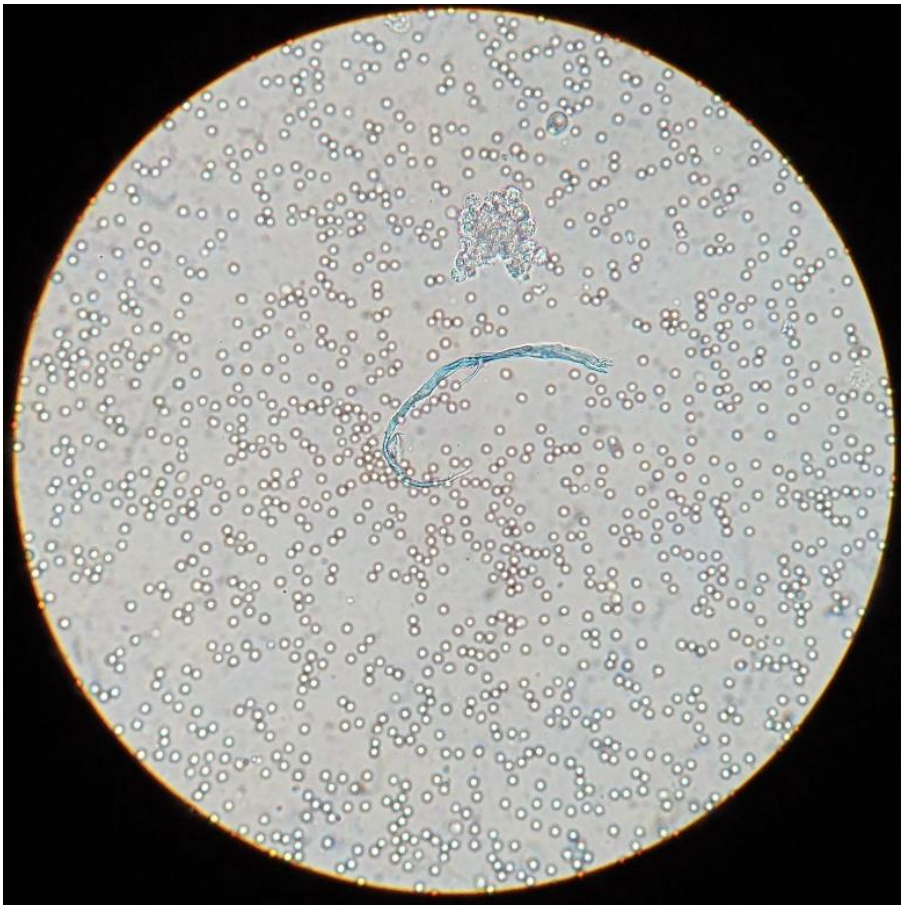

Figure 24 A fiber contaminating a canine urine sample with hematuria. The contaminant is resultant of a non-sterile container (author image)

III. The counting cell chamber – Pentasquare slide for urine sediment assessment

1. A revision of the classic method

Urine sediment examination through microscopy has been used in clinical practice since the nineteen century (Perazella, 2015) and has evolved to be the third major *in vitro* diagnostic screening test (Kurup and Leich, 2012) behind serum chemistry and complete blood count (Kurup and Leich, 2012). A complete urinalysis can provide information to detect pathologies such as liver disease and diabetes mellitus trough measurement of bilirubin, glucose and ketones concentration respectively; intravascular hemolysis indicated by increased hemoglobin values and specifically through sediment microscopic findings of casts, white blood cells, bacteria and erythrocytes, which allows early detection of renal diseases before renal failure sets (Parrah *et al*., 2013).

Although an important and useful mean of diagnosis, urinalysis is underused in veterinary medicine (Parrah *et al.*, 2013) since the traditional microscopy method is seen as labor-intensive, dull, imprecise and with a wide variability (Chien *et al*., 2007). The variability seen between observers of the same urinary sediment can be explained by the lack in teaching the correct evaluation of urine sediment microscopy. This problematic is due to several factors as the supervised instruction at the microscope is time consuming and requires specialized equipment; to teach specialized and experienced staff are needed; and urine samples previously chosen and preserved are needed (Sharda *et al.*, 2015; Fogazzi *et al.*, 2007).

Errors and variable results are not only associated to the observer of the urine sediment but also with the technic used. Every step of the procedure in preparing the urine sediment can lead to variations in results, starting with the amount of urine to centrifuge, since different amounts will result in different volumes of sediment inducing the diagnostician into error when reporting the cells observed. The volume of sediment formed is also determined by the type of centrifuge tubes used and the centrifuge itself, once the speed of centrifugation doesn't reflect the centrifugal force for it's dependent of the length of the centrifuge arm. If the centrifugal force (and time) is not taken into account it can lead to artifacts in cells, casts and other elements resulting of packing of elements at the bottom of the tube when high centrifugal forces are used, on the contrary some sediments might appear falsely inactive if the centrifugal force is not

enough to precipitate or if it causes disruption of cells (Osborne and Stevens, 1999; Carlson and Statland, 1988).

The elements identified by microscopy are highly influenced by the supernatant that remains in the tube used to resuspend the sediment, as well as by the resuspension technic (Kouri *et al.*, 2003). A large volume of supernatant will dilute the elements, giving the false idea of lesser concentration of elements. A poorly resuspended sample will adulterate the clinicians' diagnosis since heavy elements as casts remain at the bottom of the centrifuged tube, on the other hand if the resuspension is performed in a bearish way the elements might be disintegrated (Osborne and Stevens, 1999).

The most used technic of urine sediment observation is by placing a drop of the resuspended sediment on a clean microscopic slide and cover it with a clean coverslip. A standardized volume of urine sediment should be analyzed under microscope, not only because the volume affects the reported number of elements per milliliter of urine, but also an inappropriate amount of sample under the cover glass could lead to a rapidly dried sample, which will alter the morphological appearance of elements, or a floating coverslip, leading to overlapping of elements, distorting the results. A first scan of the entire field should be performed once this technic has been reported not to have a homogeneous distribution of elements, once heavier elements as casts and crystals tend to accumulate at the edges of the coverslip (**Figure 25**). Counting individual elements on 10 to 15 fields of high power fields should follow (Osborne and Stevens, 1999).

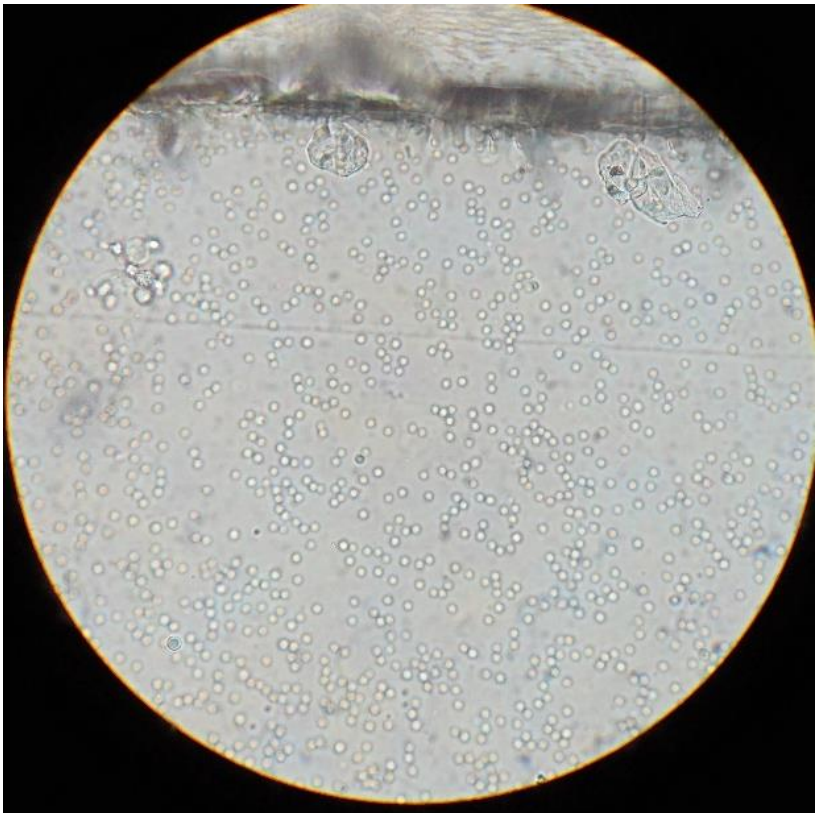

Figure 25 An example of a urine sediment prepared by the traditional method where at the edges of the coverslip there's an accumulation of heavier cells as squamous and transitional epithelial cells (author image)

Although a highly used and convenient technic the traditional method of urine sediment observation is imprecise and it's reproducibility is dependent of the material and technic used to prepare the sediment and is highly dependent from the observer (Osborne and Stevens, 1999).

2. The fast-read Pentasquare slide

The pentasquare slide (**Figure 26**) is based on the same principal as other counting cell chambers, as Neubauer and Bürker, consisting on a plastic slide with ten chambers, all with the same area, thus, when filled, the volume of urine per chamber is always the same. Due to the consistent volume of the chambers, the reported number of elements in urine sediment is determined, through appropriate calculations, per mL present in a sample of urine (F.L. Medical, 2009). This method, since is using a determined volume, provides reproducible results, even if used by different users, which doesn't happen with the traditional method, where the elements are reported by HPF (objective of 40x) or LPF (objective of 10x) regardless of the size of the sample drop or the size of the coverslip (Osborne and Stevens, 1999).

The counting cell chamber pentasquare slide ensures the precision of the sample volume inside every chamber and, consequently, the volume inside every grid allowing elements to be reported by microliter of urine avoiding errors associated with converting number of elements per HPF or LPF to number of elements per microliter. The chamber is designed to allow a quick sample examination and avoids the possibility of the sample to dry quickly, as well as ensures an homogeneous spread of urinary elements avoiding cell overcrowding (F.L. Medical, 2009).

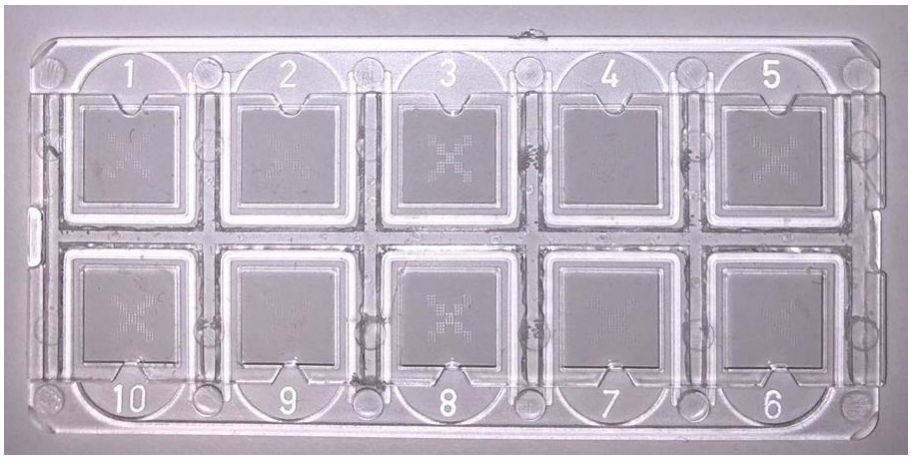

Figure 26 Pentasquare slide counting chamber with 10 chambers, each with a counting grid on its surface tube (courtesy of HV-UTAD laboratory).

3. Objectives

Urine sediment observation along with urine physical and chemical evaluation, being the third major *in vitro* diagnosis test (Chien *et al*., 2007), should be easy to perform and have a highly repeatability and reproducibility so that a patient can have a right diagnosis and evaluation of the clinical status independently of the technic used or the observer.

Thus, the main objective of this study is to determine if the counting cell chamber pentasquare slide can be used interchangeably with the traditional method of urine sediment (slide, coverslip) by evaluating its' statistical performance by method comparison studies. In addition to evaluate the pentasquare slide diagnostic accuracy it is also evaluated if the inter-observer agreement is higher in the counting cell chamber than in the traditional method as well as its repeatability. Additionally, was also the aim of the study to evaluate the performance of the pentasquare slide in the evaluation of urine sediment of dogs and cats, in an attempt to reduce the imprecisions, inconveniences and lack of reproducibility, and standardized procedures associated with the traditional method. Ultimately our aim is to validate the use of this chambers to replace the traditional method for urine sediment of slide and coverslip, in order to facilitate the urine sediment preparation and analysis in the common clinical veterinary practices.

4. Methods and materials

4.1 Population and sample collection

All samples were collected at *Hospital Veterinário da Universidade de Trás-os-Montes e Alto Douro* (HV-UTAD) between May 2nd and July 31st of 2019 for routine urinalysis purposes. Only the reminiscent of urine samples were utilized and no samples were collected specifically for this study.

All samples were collected by eco-guided cystocentesis using sterile syringe and needle. The volume of urine sample collected varied between two and five milliliters according to the size of the animal and amount of urine available for collection.

Only domestic cats (*Felis catus*) and dogs (*Canis lupus familiaris*) were included in this study. No exclusion criteria regarding the breed, sex, age or clinical status was taken. Animals were categorized in terms of age in life stages according to their status of life, in order to assess the characteristics of the population at study. Life stages in both cats and dogs were determined according not only to the age of the animal but also according to physiological and behavioral characteristics. Guidelines from the American Animal Hospital Association (AAHA) were used to classify the life stage of the animals under study (Vogt *et al.*, 2010; Creevy *et al.*, 2019; Bartges *et al.*, 2012).

4.2. Laboratorial method

The sample registration and macroscopic and chemical urine strip analysis were performed within 30 minutes after urine collection. The sediment analysis was performed within 1 and 12 hours after collection. Due to the varied range of time after collection until sediment, all the samples were refrigerated at 5ºC ± 1 ºC.

To perform the microscopic urine sediment analysis one milliliter of urine was taken from the collection syringe, after careful mixing to homogenize, into a 1.5 mL centrifuge tube. The sample is centrifuged at 2000 rpm in an MLW Centrifuge, centrifuge type TH 21 (**Figure 26**) for 5 minutes. To discard the supernatant the centrifuge tube was rapidly inverted leaving 0.1 mL of centrifuged urine at the bottom of the tube. The sediment was then gently resuspended using a 2 mL disposable pipette.

Figure 27 Centrifuge used to obtain urine sediment from the sample (courtesy of HV-UTAD laboratory)

For the sediment analysis three preparations were made: one urine sediment prepared according to the traditional method and two preparations on counting chambers.

The traditional method was prepared placing a drop of the resuspended urine sediment on a clean slide and covered with a 20 millimeter (mm) by 20 mm coverslip. The preparation was examined by two observers under microscopy. The observation started by scanning the entire field on the 10x objective to evaluate the quality of the preparation and the quantity of material present in the preparation. Following the preliminary scan a 40x objective observation was performed where the observers analyzed 10 fields counting all elements present. The results were reported by the average number of elements per HPF by both observers.

Two counting chambers were prepared for each urine and each chamber filled with resuspended urine sediment and posteriorly observed by two people. A chamber has a grid with five large squares, each one divided in nine smaller squares (**Figure 27**). The elements were reported by counting the number of elements on 10 random small squares, two in each larger square, as shown in figure 28 for an example, and their average number was reported by each observer. The average number of elements per small square was then converted in number of elements per microliter of urine.

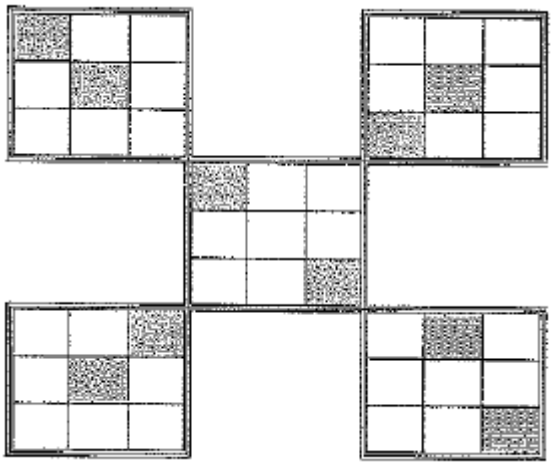

Figure 28 Representative grid of the pentasquare slide counting chamber (adapted from F.L. Medical, 2009).

In all preparations the following elements were reported by both observers, when present: erythrocytes, leucocytes, struvite, calcium oxalate and amorphous crystals, fat droplets, spermatozoids, squamous epithelial cells and granular casts.

The results were all converted into the same units of elements per microliter to allow the analysis and comparison of the results of the same urine between both methods. Different equations were used to make the conversion of values.

To calculate the number of elements per microliter in the traditional method of urine sediment the microscope field of view should be known, in this case the field of view of the 40X magnification objective is 0.45mm. The area of the field of view was calculated by multiplying 3.14 by the square radius. To know the number of HPF per milliliter the coverslip area (400 mm) was divided by the field of view area and these were divided by the product of the residual urine volume resuspended multiplied by the total volume of urine centrifuged. The

number of elements per microliter was calculated multiplying the average number of elements per HPF by the number of HPF per mL and these multiplied by 10^{-3} (Sink and Weinstein, 2012).

$$Field\ of\ view\ area = \pi * radius^2 \leftrightarrow$$
$$\leftrightarrow 3.14 * 0.225^2 = 0.159$$

$$hpf\ per\ mL = \frac{400/0.159}{0.1 * 1} = 25157$$

$$Elements\ per\ \mu L = (nr\ of\ elements\ per\ hpf * 25157) * 10^{-3}$$

The elements counted in the counting chamber should also be converted from number of elements per small square to number of elements per microliter. The formula used for the conversion was independent from the field of view from different microscopes, depending on the concentration factor which translates the volume of urine centrifuged by the residual volume of urine used for the resuspension of the sediment. Another value was used in the formula that changes only between different counting chambers, thus, if the same model of counting chambers is used at all times by the observers the factor to obtain one microliter will be constant. This constant was the factor needed to obtain 1 microliter of sample, volume that was present in each of the larger square. The formula used to convert the number of elements observed in each small square to microliter is shown below (F.L. Medical, 2009).

$$Concentration\ factor = \frac{volume\ of\ centrifuged\ urine}{residual\ volume\ of\ urine} = 10$$

$$Factor\ to\ obtain\ 1\ \mu L = \frac{volume\ of\ a\ large\ square}{volume\ of\ a\ small\ square} = \frac{0.1}{0.011} = 9$$

$$Number\ of\ elements\ per\ \mu L = \frac{average\ number\ of\ cells\ per\ small\ square * 9}{10}$$

5. Statistical Analysis

The statistical analysis was performed using JMP version 13.2.0 (SAS Institute Inc.) and Microsoft Office Excel 365 ProPlus (Microsoft Corp.) with Analyse-it for Microsoft Excel version 5.20.4 (Analyse-it Software, Ltd.).

Red blood cells, white blood cells and squamous epithelial cells were registered and analyzed as quantitative and semi-quantitative parameters by the Passing-Bablock regression. RBC and WBC were categorized in six ranges as shown in **table 1**. Other elements as crystals, fat droplets and spermatozoids where categorized as qualitative parameters and analyzed as such by evaluating the clinical performance of the counting cell chambers using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Cohen's kappa value.

For all results obtained, a value of $p < 0.05$, was considered statistically significant.

Table 1 Corresponding range in which the number of red blood cells and white blood cells are inserted, according to clinical relevance as presented by Cho and collaborators. Between parenthesis are the corresponding number of cells that are reported in the traditional method of slide and coverslip used widely for urine sediment.

RBC/WBC per uL (per HPF)	**0 – 50 (0 – 2)**	**75 – 126 (3 – 5)**	**151 – 252 (6 – 10)**	**277 – 503 (11 – 20)**	**528 – 1258 (21 – 50)**	**>1258 (>50)**
RBC/WBC range	0	1	2	3	4	5

The Passing-Bablock regression equation is presented as $y = a + bx$ where a represents the interception and b the slope of the regression line revealing constant and proportional difference and their respective confidence intervals (CI) of 95%. When the comparison of two methods results in a regression equation of $y = 0 + 1x$ a conclusion that both methods are perfectly equivalents. Thus, if the 95% confidence limit of the slope includes the value one a conclusion that the obtained slope value is not significantly different than one can be withdrawn and that there is no proportional difference between methods. No constant difference between

methods exists when in the 95% confidence interval for the intercept value zero is included since if this is the condition the intercept value obtained is not significantly different from the value zero. Achieving this two premises allows the conclusion that there is no significantly difference between methods and that both can be used interchangeably (Bilic-Zulle, 2011).

Sensitivity, specificity, positive and negative predictive values and kappa values were determined for qualitative traits. Sensitivity represents the ability of a test to correctly identify a pathology or pathological situation in patients with the disease at study. It's the quotient between the true positives and the sum of true positives and false negatives. On the other hand, the quotient between true negatives and the sum of true negatives and false positives represent the specificity of a test, this means, the ability to correctly identify the patients without the disease. Ideally, a test would have high sensitivity and high specificity. The positive and negative predictive values of a test gives the proportion of patients with truly positive and negative results within the ones diagnosed as positive and negative respectively (Lalkhen and McCluskey, 2008). In order to correlate the different groups and assessing the agreement rate between them, the Kappa value was calculated according the criteria of Landis and Koch. Kappas' value varies in a range from 0 to 1 where 0 represents the lower agreement rate opposed to the perfect correlation and agreement when the value is 1, as represented on table 2 (Landis and Koch, 1977).

Table 2 Adaptation of Landis and Koch table for agreement measure for categorical data. Negative Kappa values are possible to appear when assessing the agreement rate of a proposed test meaning that the variables have an even lower agreement strength that would be expected.

Kappa Value	Agreement Rate
< 0.00	Poor
0,00 – 0,20	Slight
0,21 – 0,40	Fair
0,41 – 0,60	Moderate
0,61 – 0,80	Substantial
0,81 – 1,00	Almost Perfect

IV. Results

1. Animals and Samples

Type II urinalysis was performed in 34 samples. These samples were collected from a population in which 50% were dogs and 50% were cats. Of the total population of dogs 8 were males (47,06%) and 9 were females (52,94%), as for the cats 11 were males (64,71%) and 6 were females (35,29%) (**Figure 29**). Considering both species under study the majority of the population had an age included in the adult life stage or older, on **Figures 30** and **31** it is represented the distribution of animals per life stage of dogs and cats, respectively.

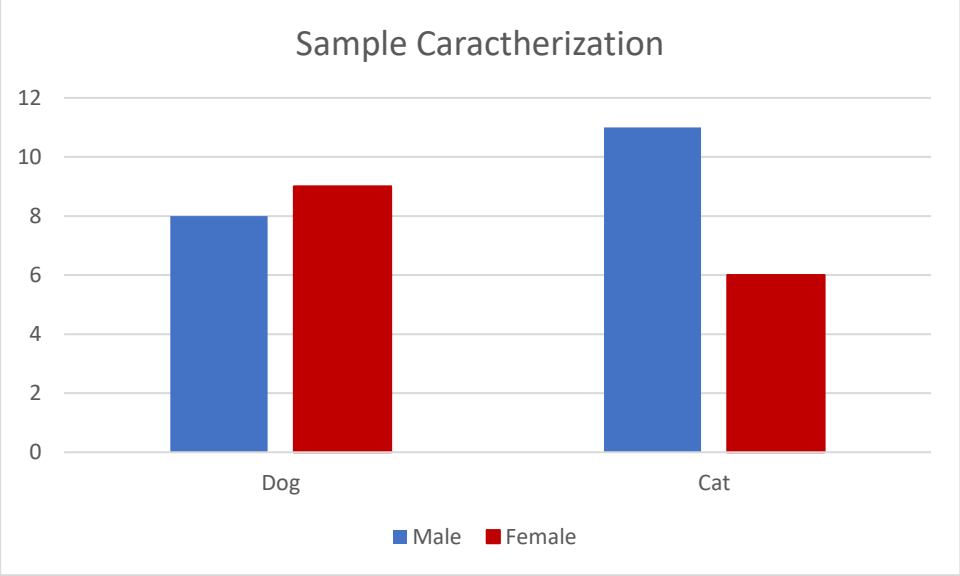

Figure 29 Graphical representation of the population sample in study for validation of pentasquare slide chambers for urine sediment. The samples in study belonged to 8 male and 9 female dogs and 11 male and 6 female cats.

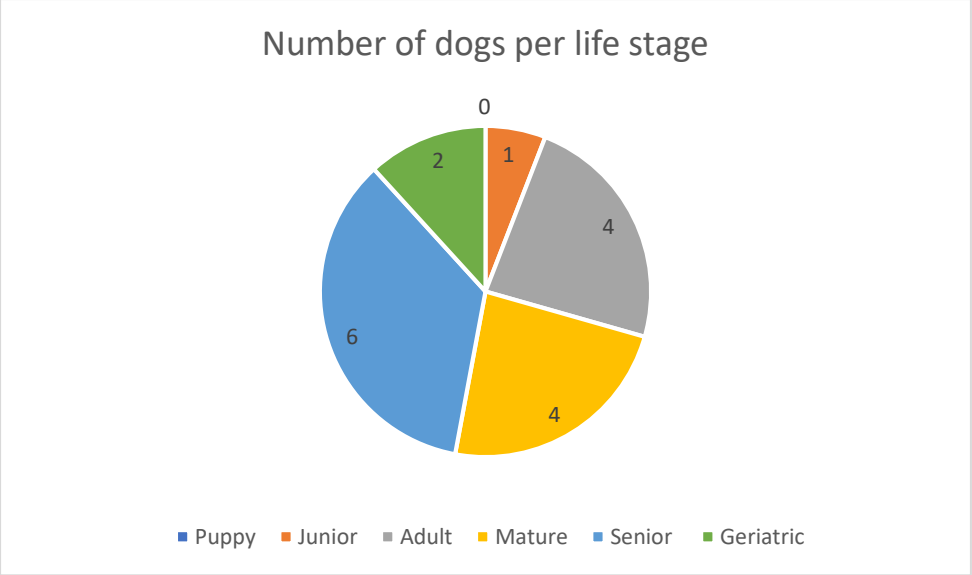

Figure 30 Number of dogs per life stage

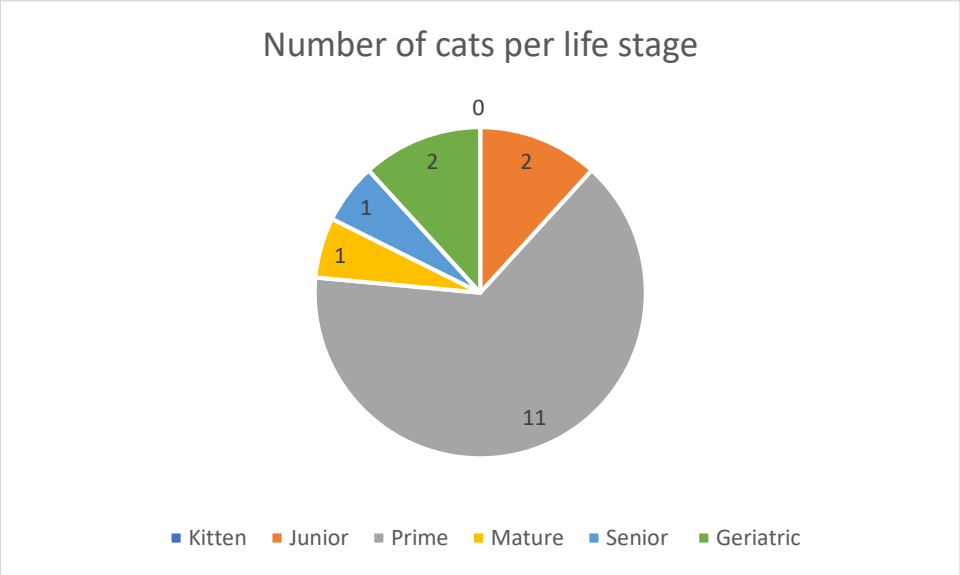

Figure 31 Number of cats per life stages

Of the 34 samples, four were excluded from the study due to highly contaminated samples or lack of information about the patient. The analysis of urine and urine sediment was performed in a total of 30 samples, 15 from cats and 15 from dogs, with and without urinary or kidney pathology, as diagnostic or evaluation of the evolution of a pathology.

2. Data Analysis

The descriptive analysis of the data was performed on all the collected data from the samples included in this study.

2.1. Comparison of quantitative parameters using the traditional method for urine sediment and the counting cell chamber

The quantitative parameters in this study (erythrocytes, leucocytes and squamous epithelial cells) were analyzed using the Passing-Bablock regression. The traditional method (method of reference) is shown in X axis of the graphic and the proposed new method (the fast-read counting cell chamber – Pentasquare) is represented in the Y axis. On **Figures 32**, **33** and **34** and **table 3** are represented the Passing-Bablock regression equations obtained from the analysis of 30 samples of urine from dogs and cats. Each sample was analyzed by the traditional method of urine microscopy and the counting cell chamber (Pentasquare slide). For RBC, WBC and SQEP cells the value of the interception is zero, and in all the 95% confidence interval limits the value zero is included. The slope is 0,78, 0,63 and 0,86 for erythrocytes, leucocytes and squamous epithelial cells respectively. All 95% confidence interval limits, for the slope values, include the value one. Bias (mean difference) for red blood cells is ±17, -5,4 for leucocytes and -2 for squamous epithelial cells (**Figures 35, 36 and 37**).

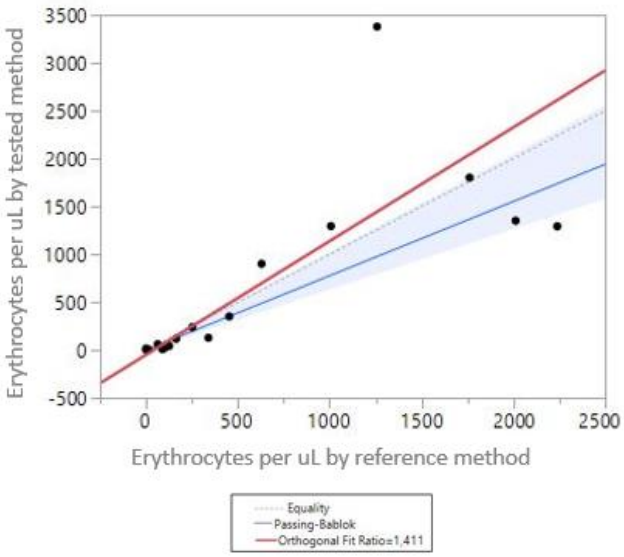

Figure 32 Representation of Passing-Bablock regression equations for red blood cells.

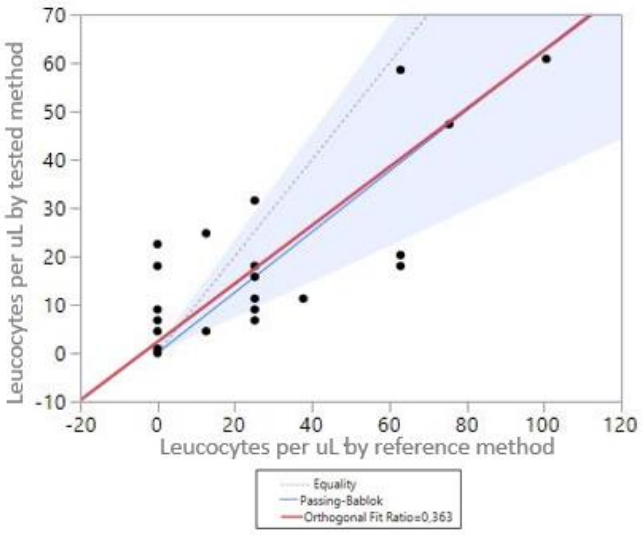

Figure 33 Representation of Passing-Bablock regression equations for white blood cells.

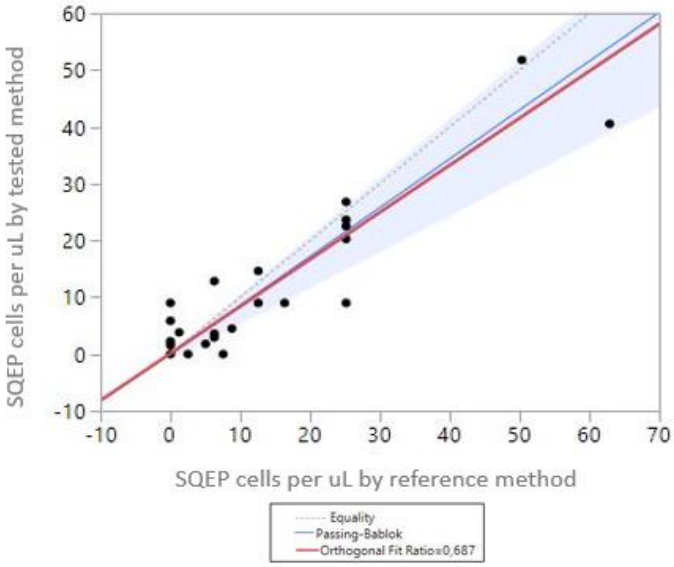

Figure 34 Representation of Passing-Bablock regression equations for squamous epithelial cells

Table 3 Representation of slope and intercept values obtained by the Passing-Bablock regression, values of bias obtained by the Bland-Altman plot and correlation values

	RBC per uL	WBC per uL	SQEP cells per uL
Slope (95% CI)	0,78 (0,63 – 1,03)	0,63 (0,37 – 1,10)	0,86 (0,64 – 1,03)
Intercept (95% CI)	0 (0 – 0)	0 (0 – 1,44)	0 (-1,13 – 0,25)
Bias (95% CI)	16,81 (-153,84 – 187,45)	-5,368114 (-11,72 – 0,98)	-1,968412 (-4,27 – 0,33)
Correlation	0,80	0,82	0,92

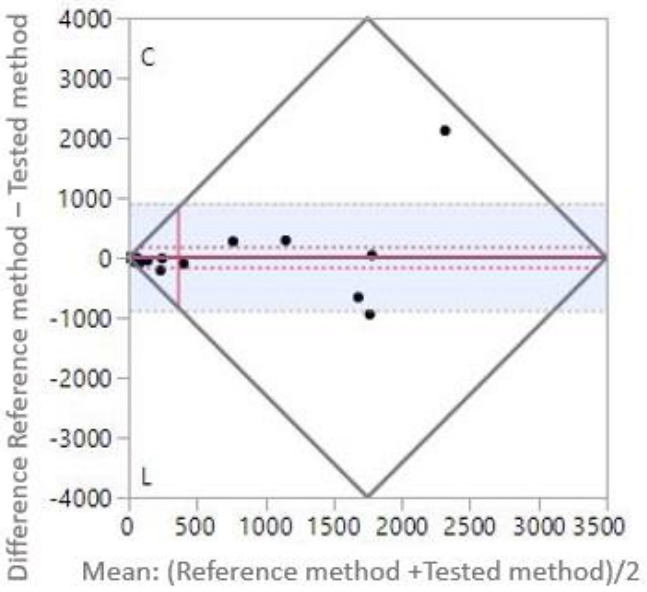

Figure 35 Representation of a Bland-Altman scatter plot of erythrocytes per uL. In the graphic are represented the values of mean difference and upper and lower 95% confidence interval limits of the mean difference (bias).

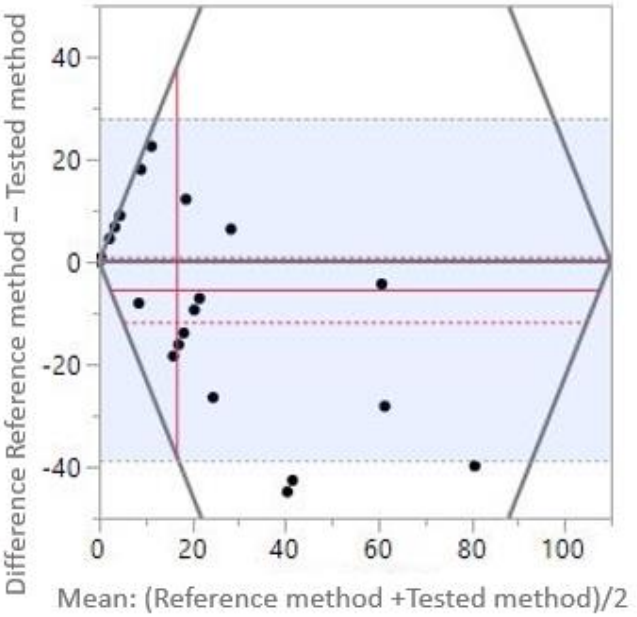

Figure 36 Representation of a Bland-Altman scatter plot of erythrocytes per uL. In the graphic are represented the values of mean difference and upper and lower 95% confidence interval limits of the mean difference (bias).

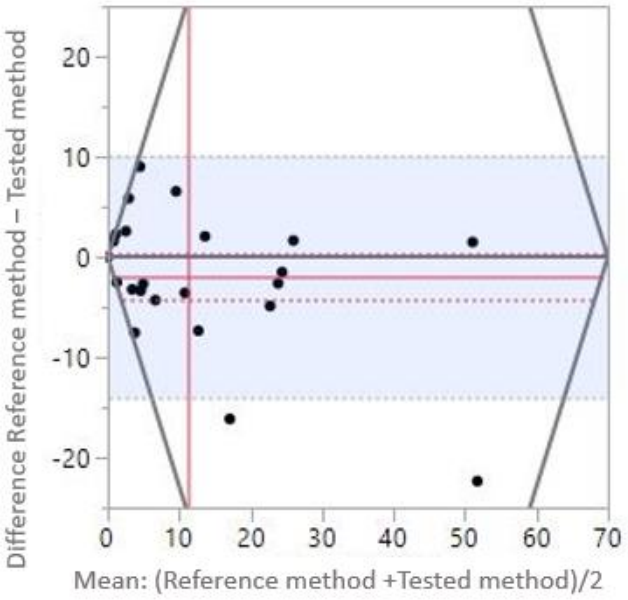

Figure 37 Representation of a Bland-Altman scatter plot of squamous epithelial cells per uL. In the graphic are represented the values of mean difference and upper and lower 95% confidence interval limits of the mean difference (bias).

2.2. Comparison of qualitative parameters using the traditional method for urine sediment and the counting cell chamber

Table 4 summarizes the diagnostic accuracy of the counting cell chamber (pentasquare) when compared to the reference traditional method of urine sediment microscopy for qualitative parameters: Struvite, Calcium oxalate and amorphous crystals, fat droplets, spermatozoids and granular casts. Sensitivity for crystals has values from 42,9% for amorphous crystals to 83,3% for struvite crystals, being that for calcium oxalate monohydrated crystals the sensitivity of method being tested is 50%. Granular casts presented a sensitivity of 66,7% when compared to the reference method. For fat droplets the reported sensitivity is of 84,2% and of 66,7% for spermatozoids.

Table 4 Diagnostic accuracy of the counting cell chamber when compared to the reference method of traditional urine sediment preparation for qualitative parameters. Sensitivity, specificity, PPV (positive predictive value), NPV (negative predictive value) and Cohen's Kappa value with respective CI (confidence interval) for each parameter. Only the qualitative parameters presented on this table were reported on the urine sediment analysis of the population sample.

	Struvite Crystals	Calcium oxalate crystals	Amorphous crystals	Granular casts	Fat droplets	Spermatozoids
Sensitivity	83,3%	50,0%	42,9%	66,7%	84,2%	66,7%
Specificity	95,2%	99,0%	100%	97,8%	89,1%	97,6%
PPV	78,9%	66,7%	100%	80,0%	82,6%	85,7%
NPV	96,4%	98,0%	72,1%	95,7%	90,1%	93,2%
Kappa (95% Cl)	0,77 (0,61 – 0,93)	0,56 (0,11 – 1,00)	0,47 (0,31 – 0,63)	0,69 (0,47 – 0,92)	0,73 (0,59 – 0,87)	0,70 (0,51 – 0,90)

Overall, for all qualitative parameters the counting chamber revealed a high specificity value. Crystals show a specificity of 95,2%, 99.0% and 100% for struvite, calcium oxalate and amorphous crystals respectively. Granular casts were identified with a specificity of 97,8%, fat droplets with a specificity of 89,1% and spermatozoids of 97,6%.

The lower registered positive predictive value was 66,7% for calcium oxalate monohydrated crystals and a higher value of 100% for amorphous crystals. As for the lower and higher values registered for negative predictive value were 72,1% and 98,0% respectively. Kappa values revealed an agreement rate of the qualitative parameters of moderate to substantial.

2.3. Counting cell chamber pentasquare slide repeatability and inter-observer agreement

The reproducibility and repeatability of the counting cell chambers are represented in **tables 5 and 6** where the correlation between observers within the same preparation and the correlation between preparations for the same sample are shown.

Table 5 Reproducibility represented by the inter-observer agreement between the two observers in the traditional method preparation and in both preparation on the Pentasquare slide

	Traditional method	Pentasquare Slide (1)	Pentasquare Slide (2)
Erythrocytes	97,16%	99,70%	99,24%
Leucocytes	72,50%	87,11%	89,45%
SQEP	96,04%	85,07%	92,45%
Struvite Crystals	87,39%	84,61%	86,77%
Calcium Oxalate Crystals	-4,00%	0	0
Amorphous Crystals	84,52%	100%	100%
Granular Casts	34,75%	67,57%	68,92%
Fat Droplets	100%	100%	100%
Spermatozoids	100%	100%	61,11%

Table 6 The repeatability of the pentasquare slide is presented in this table. The values presented indicate the correlation between the observed elements by the same operator on two preparations of the same sample

	Correlation
Erythrocytes	97,28%
Leucocytes	92,63%
SQEP	92,53%
Struvite Crystals	80,40%
Calcium Oxalate Crystals	69,94%
Amorphous Crystals	100%
Granular Casts	68,26%
Fat Droplets	95,55%
Spermatozoids	82,48%

V. Discussion

In veterinary medicine urinalysis is the third major *in vitro* screening test, right behind complete blood count and serum chemistry (Kurup and Leich, 2012). A well performed urinalysis provides a direct indication of the renal and genitourinary systems health status and is used as a good diagnosis and treatment response control tool (Chien *et al.*, 2007). However it has become an underused test in veterinary practice (Parrah *et al.*, 2013) since the 1920's when it suffered a progressive decline (Fogazzi and Garigali, 2003) due to lack of appropriate resources such as trained professionals, time and equipment (Becker *et al.*, 2016).

Automated urinalysis has been introduced for almost two decades (Wang *et al.*, 2019) but most clinicians don't consider it the election method because, even though it shows results with higher accuracy and reliability and has advantages as reduced labor, time and potential variations, also presents some major limitations as lack of precision, standardization and deficits of sensitivity and specificity (Lee *et al.*, 2016). In addition to the previous limitations to automated urinalysis most of the practitioners don't have an automated analyzer in house due to its high costs. For these reasons the manual microscopy remains the gold standard (Cho *et al.*, 2019; Yüksel *et al.*, 2013) in veterinary clinical practice.

Traditional manual microscopy of urine sediment is still used among general practitioners since it needs little material, is almost inexpensive and remains an important tool to evaluate renal and urologic disease (Perazella *et al.*, 2008). However, this technic is associated with high imprecision and wide variability (Chien *et al.*, 2007) in addition to the results being reported in number of particles per HPF or LPF. As previously approached, the number of particles per HPF and LPF it's highly dependent on various factors (Wang *et al.*, 2019) as the area of the coverslip and field of microscopic view (Sink and Weinstein, 2012). Due to the need for a more reliable and easy method of urine sediment analysis cell counting chambers were introduced and are already being used in human medicine as reference method (Wang *et al.*, 2019).

Cell counting chambers are a standardize, low-cost alternative to the traditional method of urine sediment. Advantages of counting cell chamber, most of them already described previously, are the precision of sample volume inside every chamber, a more homogeneous distribution of particles, reduces rate of sample drying and the reduced number of glass slides to be prepared (F.L. Medical, 2009). Taking into account the advantages of cell counting

chamber (pentasquare) this study was performed in order to analyze the diagnostic accuracy of the chambers when compared to the "gold standard" technic in veterinary medicine - the traditional method of urine sediment analysis. Although several limitations are pointed out to the traditional method of urine sediment analysis, the mounted slide and coverslip is still the reference method of sediment evaluation and therefore the selected method for comparison in this study.

The validation of the cell counting chambers was made by evaluating its performance using a proper statistical analysis for method comparison studies. Bland-Altman scatter plots and Passing-Bablock regression were performed to assess the comparability of the two methods in study: traditional urinary sediment analysis versus cell counting chamber for urine sediment (Simundic, 2016).

Passing-Bablock regressions were calculated for the quantitative elements: red blood cells, white blood cells and squamous epithelial cells. The intercept for all regressions has a value of zero. As well, all regressions intercept 95% confidence interval contains the value zero. These results of interceptions demonstrate that there is no constant differences between the reference method of urine sediment and the tested one. All slope values have a 95% confidence interval that includes the value one, thus it can be concluded that slope value are not significantly different than the value one, so we can conclude that there is no proportional difference between the two methods (Bilic-Zulle, 2011). For quantitative parameters the counting cell chamber (pentasquare slide) can be used interchangeably with the traditional reference method.

Bias was also calculated for quantitative parameters and their value reflect the ratio in which both methods differ. A bias exists between both methods, allowing to assess how the values measured by the tested method differ from the values obtained from the mean difference of methods in study. However, this bias doesn't seem to be clinically relevant. The fact that the 95% confidence interval limits for bias include the value zero determines that the values of bias for either quantitative parameters are not statistically significant (Hanneman, 2008). Still, for more accurate results of bias and a narrower corresponding 95% confidence interval limits, a larger population and higher number of samples is required.

For qualitative parameters (crystals, fat droplets and spermatozoids), the diagnostic accuracy of the counting cell chamber was assessed by evaluating the sensitivity, specificity, PPV (predictive positive value), PNV (predictive negative value) and agreement rate. The sensitivity has a high value, approximately 67%, for all quantitative parameters, which indicate a great capacity for correctly identify the patients that exhibit the pathology (Lalkhen and McCluskey, 2008). Amorphous crystals are the exception for this high sensitivity of the counting cell chamber, for these crystals the calculated sensitivity is only of 43%, most likely due to the small number of samples that presented amorphous crystals. On the other hand, the specificity, the capacity of correctly identify the absence of the elements, on amorphous crystals is 100%. All other qualitative parameters also showed values of specificity higher than 90%. These values of high sensitivity and high specificity indicate that the counting cell chamber is a highly accurate test (Lalkhen and McCluskey, 2008).

The predictive values were calculated for all observed crystals, fat droplets, granular casts and spermatozoids. Unlike sensitivity and specificity, PPV and NPV are dependent on the population under study and are influenced by the prevalence of the diseases (Lalkhen and McCluskey, 2008). Positive predictive value is higher for amorphous crystals, although it presents values higher than 60% for all remaining elements. On the contrary, the negative predictive value is lower for amorphous crystals (about 72%). The counting cell chamber presents high negative predictive values for all remaining elements. These results indicate a high capacity for the chamber to identify true positive and true negative patients regarding the presence or absence of the elements in study in urine sediment. Unfortunately, the absence of similar studies in veterinary medicine, prevent us to better discuss present results.

Inter-observer variability and disagreements have been pointed out as some of the imprecisions of the traditional method for urine sediment analysis (Chien *et al.*, 2007; Chan and Szeto, 2004). Thus, this study evaluates the correlation between the observations performed by the two observers in urine sediment preparations: one preparation of the traditional method and two preparations of the pentasquare slide. For erythrocytes, leucocytes and amorphous crystals the counting cell chamber pentasquare slide presents higher correlations between observers in comparison with traditional method. For squamous epithelial cells and struvite crystals the traditional method presents slightly better agreement between observers than the pentasquare slide even so the values of observers' correlation are very similar between methods. The observation of fat droplets and spermatozoids has perfect correlation between observers in both

methods apart from the replication of the pentasquare slide for spermatozoids that presented a lower value of correlation probably associated with errors associated with the preparation or analysis of the counting cell chamber (Osborne and Stevens, 1999). For calcium oxalate crystals the values of inter-observer correlation are null, and the most probable explanation is the low count and number of samples with these crystals (N = 3) (Hanneman, 2008). Overall the pentasquare slide presents high correlation between observers which indicates a good reproducibility of urine sediment samples on the counting cell chamber in study in agreement to other studies performed in human medicine (Wang *et al.*, 2019)

Regarding repeatability between the two pentasquare slide preparations for the same case, high values of correlation were found as demonstrated in **table 6**. These high values indicate an almost perfect repeatability of observations when more than one preparation of the same sample is made. This was the first time, to the best of authors' knowledge that a similar study is done in veterinary medicine, so we have no possibilities for comparison with other works.

The present study has some limitations. The first one was the relatively low number of samples which can lead to a small variability of elements reported. In some cases, the number of samples that had a determined element is too small to correctly calculate its statistical performance, as it happened for calcium oxalate crystals for example. The statistical analysis would have been better if the number of fields in the traditional method or the number of small squares in the counting cell chamber counted had have been higher, however it is noteworthy to note that we followed the literature recommendations with respect number of observers and number of observations in each method (F.L. Medical, 2009; Osborne and Stevens, 1999; Hanneman, 2008; Bilic-Zulle, 2011; Landis and Koch, 1977).

Further studies are needed to assess the diagnostic accuracy of the counting cell chamber – pentasquare slide – for every element that might be seen in urine sediment as well to evaluate if the reproducibility and repeatability maintain the tendency when the number of samples is larger. Even though it was not explored in this study, the pentasquare slide instructions indicate that these chambers can be used to analyze non-centrifuged urine, thus, further studies should be performed to evaluate this feature that would significantly decrease the time and labor needed to prepare the urine sediment.

VI. Conclusions

This study evaluated the performance of the counting cell chamber – pentasquare slide– on its diagnostic accuracy in veterinary medicine on a daily basis veterinary practice.

According to the statistical method comparison analysis the pentasquare slide can be used interchangeably with the traditional method of urine sediment, having a high sensitivity, specificity and agreement rate when compared to the actual reference method. Along with these findings the statistical analysis also verified a high reproducibility and repeatability which guarantees similar results between different preparations of the same sample as well as similar results when analyzed by different observers.

VII. Bibliography

Albasan, H. *et al.* (2003) 'Effects of storage time and temperature on pH, specific gravity, and crystal formation in urine samples from dogs and cats.', *Journal of the American Veterinary Medical Association*, 222(2), pp. 176–9.

Alleman, R. and Wamsley, H. (2017) 'Complete Urinalysis', in *BSAVA Manual of Canine and Feline Nephrology and Urology*, pp. 60–83.

Athanasiou, L. V. *et al.* (2018) 'Comparison between the urine dipstick and the pH-meter to assess urine pH in sheep and dogs', *Veterinary Clinical Pathology*, 47(2), pp. 284–288.

Bartges, J. *et al.* (2012) 'AAHA canine life stage guidelines', *Journal of the American Animal Hospital Association*, 48(1), pp. 1–11.

Bartges, J. W. (2009) 'Hematuria and Other Conditions Causing Discolored Urine', in *Textbook of Veterinary Internal Medicine, 7th Edition*, pp. 655–659.

Bartges, J. W. (2016) 'Feline Calcium Oxalate Urolithiasis: Risk factors and rational treatment approaches', *Journal of Feline Medicine and Surgery*, 18(9), pp. 712–722.

Bartges, J. W. and Callens, A. J. (2015) 'Urolithiasis', *Veterinary Clinics of North America - Small Animal Practice*, 45(4), pp. 747–768

Batamuzi, E. K. and Kristensen, F. (1995) 'Diagnostic importance of urothelial cells of the dog and cat', *Journal of Small Animal Practice*, 36(1), pp. 17–21

Bauer, N., *et al.* (2008) 'Evaluation the Clinitek status^{TM} automated dipstick analysis device for semiquantitative testing of canine urine', *Research in Veterinary Science*, 85(3), pp. 467–472.

Becker, G. J., Garigali, G. and Fogazzi, G. B. (2016) 'Advances in urine microscopy', *American Journal of Kidney Diseases*. Elsevier Inc, 67(6), pp. 954–964.

Bilic-Zulle, L. (2011) 'Comparison of methods: Passing and Bablock regression', *Biochemia medica*, 21(3), pp. 49–52.

Brons, A. K. *et al.* (2013) 'SLC3A1 and SLC7A9 mutations in autosomal recessive or dominant canine cystinuria: A new classification system', *Journal of Veterinary Internal Medicine*, 27(6), pp. 1400–1408.

Brown, S. A. (2011) ‘Physiology of the kidneys’, in *Nephrology and Urology of Small Animals*, pp. 10–17.

Byron, J. K. (2019) ‘Urinary Tract Infection’, *Veterinary Clinics of North America - Small Animal Practice*. Elsevier Inc, 49(2), pp. 211–221.

Caleffi, A. and Lippi, G. (2015) ‘Cylindruria’, *Clinical Chemistry and Laboratory Medicine*, 53, pp. S1471–S1477.

Callens, A. J. and Bartges, J. W. (2012) ‘Urinalysis’, *Imaging and Technology in Urology: Principles and Clinical Applications*, 9781447124, pp. 127–130.

Carlson, D. A. and Statland, B. E. (1988) ‘Automated urinalysis’, *Clinics in Laboratory Medicine*, 8(3), pp. 449–461.

Cavanaugh, C. and Perazella, M. A. (2019) ‘Urine Sediment Examination in the Diagnosis and Management of Kidney Disease: Core Curriculum 2019’, *American Journal of Kidney Diseases*. Elsevier Inc, 73(2), pp. 258–272.

Chan, R. W. Y. and Szeto, C. C. (2004) ‘Advances in the clinical laboratory assessment of urinary sediment’, *Clinica Chimica Acta*, 340(1–2), pp. 67–78.

Chandrashekar, V. (2018) ‘Improved contrast microscopy: modification of bright field for urine sediment visualisation’, *Diagnosis (Berlin, Germany)*, 5(1), pp. 29–34

Chawla, L. S. *et al.* (2008) ‘Urinary sediment cast scoring index for acute kidney injury: A pilot study’, *Nephron - Clinical Practice*, 110(3), pp. 1 - 7

Chew, D. *et al.,* (2011) *Canine and Feline Nephrology and Urology*, pp. 1 - 31

Chien, T. I. *et al.* (2007) ‘Urine sediment examination: A comparison of automated urinalysis systems and manual microscopy’, *Clinica Chimica Acta*, 384(1–2), pp. 28–34.

Cho, J. *et al.* (2019) ‘Comparison of five automated urine sediment analyzers with manual microscopy for accurate identification of urine sediment’, pp. 1 - 13

Creevy, K. E. *et al.* (2019) ‘2019 AAHA Canine Life Stage Guidelines’, *Journal of the American Animal Hospital Association*, 55(6), pp. 267–290.

Devuyst, O., Dahan, K. and Pirson, Y. (2005) ‘Tamm-Horsfall protein or uromodulin: New ideas about an old molecule’, *Nephrology Dialysis Transplantation*, 20(7), pp. 1290–1294.

Dorsch, R., *et al.* (2019) 'Urinary tract infection and subclinical bacteriuria in cats: A clinical update', *Journal of Feline Medicine and Surgery*, 21(11), pp. 1023–1038.

F.L. Medical (2009) 'Pentasquare Slide: Instructions for use', pp. 1- 9

Fogazzi, G. B. *et al.* (2007) 'How to improve the teaching of urine microscopy', *Clinical Chemistry and Laboratory Medicine*, 45(3), pp. 407–412.

Fogazzi, G. B. *et al.* (2012) 'Urinary sediment findings in acute interstitial nephritis', *American Journal of Kidney Diseases*. Elsevier Inc., 60(2), pp. 330–332.

Fogazzi, G. B. and Delanghe, J. (2018) 'Microscopic examination of urine sediment: Phase contrast versus bright field', *Clinica Chimica Acta*, 487(October), pp. 168–173.

Fogazzi, G. B. and Garigali, G. (2003) 'The clinical art and science of urine microscopy', *Current Opinion in Nephrology and Hypertension*, 12(6), pp. 625–632.

Fogazzi GB (1996) 'Crystalluria: a neglected aspect of urinary sediment analysis', *Nephrology Dialysis Transplantation*, 11, pp. 379–87.

Forrester, S. D. (2004) 'Diagnostic approach to hematuria in dogs and cats', *Veterinary Clinics of North America - Small Animal Practice*, 34(4), pp. 849–866.

George, J. W. (2001) 'The Usefulness and Limitations of Hand-held Refractometers in Veterinary Laboratory Medicine: An Historical and Technical Review', *Veterinary Clinical Pathology*, 30(4), pp. 201–210.

Gizzarelli, M. *et al.* (2019) 'Prevalence of Proteinuria in Owned Dogs from Italy: A Multicentric Study', *Veterinary Medicine International*, 2019, pp. 1–5.

Hanneman, S. K. (2008) 'Design , Analysis , and Interpretation of Method-Comparison Studies', 77030, pp. 223–234.

Harley, L. and Langston, C. (2012) 'Proteinuria in dogs and cats', *Canadian Veterinary Journal*, 53(6), pp. 631–638.

Heiene, R. *et al.* (2010) 'Glucosuria in Norwegian elkhounds and other breeds during dog shows', *Veterinary Record*, 166(15), pp. 459–462.

Hernandez, A. M. *et al.* (2019) 'Comparison of the performance of the IDEXX SediVue Dx® with manual microscopy for the detection of cells and 2 crystal types in canine and feline urine', *Journal of Veterinary Internal Medicine*, 33(1), pp. 167–177.

Ince, F. D. *et al.* (2016) 'The comparison of automated urine analyzers with manual microscopic examination for urinalysis automated urine analyzers and manual urinalysis', *Practical Laboratory Medicine*, 5, pp. 14–20.

Jacob, F. *et al.* (2005) 'Evaluation of the association between initial proteinuria and morbidity rate or death in dogs with naturally occurring chronic renal failure', *Journal of the American Veterinary Medical Association*, 226(3), pp. 393–400.

Kanbay, M., *et al.* (2010) 'Acute tubular necrosis and pre-renal acute kidney injury: Utility of urine microscopy in their evaluation - A systematic review', *International Urology and Nephrology*, 42(2), pp. 425–433.

Kouri, T. *et al.* (2000) 'European Urinalysis Guidelines', *Scandinavian Journal of Clinical and Laboratory Investigation*, 60(sup231), pp. 1–96.

Kouri, T., *et al.*. (2003) 'ISLH recommended reference procedure for the enumeration of particles in urine', *Laboratory Hematology*, 9(2), pp. 58–63.

Kurup, R. and Leich, M. (2012) 'Comparison of urine analysis using manual method and sedimentation method', *West Indian Medical Journal*, 61(3), pp. 240 - 244

Lalkhen, A. G. and McCluskey, A. (2008) 'Clinical tests: Sensitivity and specificity', *Continuing Education in Anaesthesia, Critical Care and Pain*, 8(6), pp. 221–223.

Landis, J. R. and Koch, G. G. (1977) 'The Measurement of Observer Agreement for Categorical Data', *Biometrics*, 33(1), pp. 159–174.

Lee, W., *et al.* (2016) 'Comparison of the Automated Cobas U 701 Urine Microscopy and UF-1000i Flow Cytometry Systems and Manual Microscopy in the Examination of Urine Sediments', *Journal of Clinical Laboratory Analysis*, 30(5), pp. 663–671.

Lees, G. E. *et al.* (2005) 'Assessment and management of proteinuria in dogs and cats: 2004 ACVIM Forum Consensus Statement (Small Animal)', *Journal of Veterinary Internal Medicine*, 19(3), pp. 377–385

Mizukami, K., *et al*. (2015) 'Feline cystinuria caused by a missense mutation in the SLC3A1 gene', *Journal of Veterinary Internal Medicine*, 29(1), pp. 120–125.

Okafor, C. C. *et al.* (2019) 'Factors associated with hematuric struvite crystalluria in cats', *Journal of Feline Medicine and Surgery*, 21(10), pp. 922–930.

Osborne, C. A. *et al.* (2009) 'Analysis of 451,891 Canine Uroliths, Feline Uroliths, and Feline Urethral Plugs from 1981 to 2007: Perspectives from the Minnesota Urolith Center', *Veterinary Clinics of North America - Small Animal Practice*, 39(1), pp. 183–197.

Osborne, C. A. and Stevens, J. B. (1999) *Urinalysis: A Clinical Guide to Compassionate Patient Care*, *Veterinary Clinical Pathology*, pp. 125 - 131

Pappas, P. G. *et al.* (2018) 'Invasive candidiasis', *Nature Reviews Disease Primers*. Macmillan Publishers Limited, 4(May), pp. 1–20.

Parrah, J. D. *et al.* (2013) 'Importance of urinalysis in veterinary practice - A review', *Veterinary World*, 6(9), pp. 640–646.

Perazella, M. A. *et al.* (2008) 'Diagnostic value of urine microscopy for differential diagnosis of acute kidney injury in hospitalized patients', *Clinical Journal of the American Society of Nephrology*, 3(6), pp. 1615–1619.

Perazella, M. A. (2015) 'The Urine Sediment as a Biomarker of Kidney Disease', *American Journal of Kidney Diseases*. Elsevier Inc, 66(5), pp. 748–755.

Perazella, M. A. and Fogazzi, G. B. (2010) 'The Urinary Sediment: An Integrated View, 3rd edition', *Kidney International*. Nature Publishing Group, 78(12), pp. 1202–1203.

Piech, T. L. and Wycislo, K. L. (2019) 'Importance of Urinalysis', *Veterinary Clinics of North America - Small Animal Practice*. Elsevier Inc, 49(2), pp. 233–245.

Praga, M. and González, E. (2012) 'Acute interstitial nephritis', *Kidney International*, 77(11), pp. 956–961.

Queau, Y. (2019) 'Nutritional Management of Urolithiasis', *Veterinary Clinics of North America - Small Animal Practice*. Elsevier Inc, 49(2), pp. 175–186.

Rabinovitch, A. *et al.* (2009) 'Clinical and Laboratory Standards Institute (CLSI). Urinalysis:

Approved Guideline—Third Edition', *Clinical and Laboratory Standards Institute (CLSI)*, 29(4), pp. 648 - 653

Reagan, K. L. *et al.* (2019) 'Risk factors for Candida urinary tract infections in dogs and cats', *Journal of Veterinary Internal Medicine*, 33(2), pp. 648–653.

Reece, W. O. (2015) 'The Kidneys and Urinary System', in *Dukes' Physiology of Domestic Animals*, pp. 157–202.

Reece, W. O. and Rowe, E. W. (2017) *Functional Anatomy and Physiology of Domestic Animals*, pp. 298 - 331

Reine, N. J. and Langston, C. E. (2005) 'Urinalysis interpretation: How to squeeze out the maximum information from a small sample', *Clinical Techniques in Small Animal Practice*, 20(1 SPEC.ISS.), pp. 2–10.

Rizzi, T. E. *et al.* (2017) *Atlas of Canine and Feline Urinalysis*, pp. 3 - 179

Serafini-Cessi, F., Malagolini, N. and Cavallone, D. (2003) 'Tamm-Horsfall glycoprotein: Biology and clinical relevance', *American Journal of Kidney Diseases*, 42(4), pp. 658–676.

Sharda, N. *et al.* (2015) 'Manual Urine Microscopy Versus Automated Urine Analyzer Microscopy in Patients with Acute Kidney Injury', *Laboratory Medicine*, 45(4), pp. e152–e155.

Simundic, A. (2016) 'Statistical analysis in method comparison studies – Part one', *Acutecaretesting.Org*, (May), pp. 1–7.

Sink, C. A. and Feldman, B. F. (2004) *Laboratory Urinalysis and Hematology for the Small Animal Practitioner*, pp. 21 - 42

Sink, C. A. and Weinstein, N. M. (2012) *Practical Veterinary Urinalysis, pp. 55 - 112*

Tvedten, H. W. and Norén, A. (2013) 'Comparison of a Schmidt and Haensch refractometer and an Atago PAL-USG Cat refractometer for determination of urine specific gravity in dogs and cats', *Veterinary Clinical Pathology*, 43(1), pp. 63–66.

Verdesca, S. *et al.* (2007) 'How a skilful and motivated urinary sediment examination can save the kidneys', *Nephrology Dialysis Transplantation*, 22(6), pp. 1778–1781.

Vilhena, H. C. R. *et al.* (2015) 'Urine protein-to-creatinine concentration ratio in samples collected by means of cystocentesis versus manual compression in cats', *JAVMA Scientific*

Reports, 246(863). pp- 862 - 867

Vogt, A. *et al.* (2010) 'AAFP – AAHA Feline Life Stage Guidelines Background and Goals', *Journal of the American Animal Hospital Association*, pp. 70–85.

Wang, L. *et al.* (2019) 'The standardization of the report for urine cell counting—A converting factor for Sysmex UF-1000i', *Journal of Clinical Laboratory Analysis*, (August 2018), pp. 1–5.

Wolf, A., Pillay, V. (1969) 'Renal Concentration Tests', *The American Journal of Medicine*, 46(June), pp. 837–843.

Yasui, Y. *et al.* (1995) 'Urinary sediment analyzed by flow cytometry', *Communications in Clinical Cytometry*, 22(1), pp. 75–79.

Yüksel, H. *et al.* (2013) 'Comparison of fully automated urine sediment analyzers H800-FUS100 and Labumat-Urised with Manual Microscopy', *Journal of Clinical Laboratory Analysis*, 27(4), pp. 312–316.

Zeugswetter, F. K. *et al.* (2019) 'Basal glucosuria in cats', *Journal of Animal Physiology and Animal Nutrition*, 103(1), pp. 324–330.

utad
HOSPITAL VETERINÁRIO

Projeto câmaras de sedimento

Exame de urina

Proprietário:_____ Nome do animal:_____

Espécie:_____ Idade:_____ Género:_____ Raça:_____

Nº QVET: _____ Nº LPC:_____

EXAME COMPLETO DE URINA: Urina tipo I ☐ / urina tipo II ☐

Técnica de colheita: Micção espontânea ☐ Algaliação ☐ Cistocentese ☐

Aspecto macroscópico:_____ Turbidez:_____

Densidade:_____ pH:_____ Nitritos:_____ Proteínas:_____

Glicose:_____ Corpos cetónicos:_____ Urobilinogénio:_____

Bilirrubina:_____ Sangue:_____ Hemoglobina:_____

LÂMINA DE SEDIMENTO URINÁRIO

Observador 1:

Observador 2:

CÂMARA DE SEDIMENTO 1

Observador 1:

Observador 2:

CÂMARA DE SEDIMENTO 2

Observador 1:

Observador 2: