ZOETIS DIAGNOSTICS

vetscan Imagyst®

AI Urine Sediment

Medical Whitepaper

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Urinalysis: Unlocking Valuable Insights

Introduction – Urinalysis as a Part of the Minimum Database

The routine complete urinalysis is a relatively quick and inexpensive test which can be readily performed in most veterinary clinics. It is an essential part of the diagnostic evaluation of sick patients, and the results should be interpreted along with the results of a blood chemistry panel. Ideally, urine should be collected at the same time blood is collected for haematology and clinical chemistry, as part of the diagnostic minimum database.

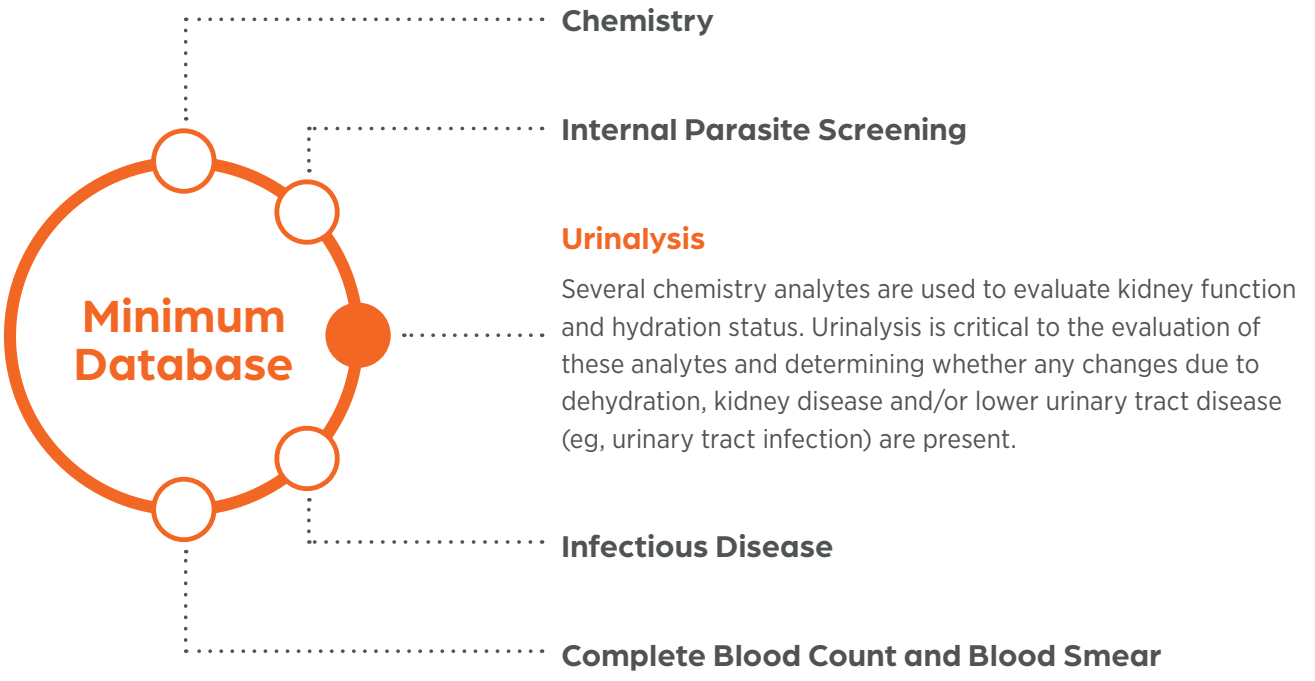


Figure 1: Diagnostic Minimum Database

Complete interpretation of a blood chemistry panel cannot be performed without concurrent knowledge of the urinalysis, particularly if there are abnormalities in renal (e.g. urea nitrogen and creatinine) or acid-base parameters. Similarly, concurrent knowledge of haematology and blood chemistry results facilitates interpretation of certain abnormalities in urine (e.g. haematuria, glucosuria, ketonuria).[1,2,3]

In addition, there may be abnormalities discovered on the urinalysis prior to evidence of disease on other testing modalities, eg. bilirubinuria prior to hyperbilirubinaemia and proteinuria prior to azotaemia in renal disease. In human medicine, it has been reported that over 30% of urine studies conducted on patients with normal general blood work results had abnormal urine sediment results, supporting the importance of urine sediment microscopic examination. Without a urine sediment exam, clinically relevant findings may go unreported.[4] Ultimately, a urinalysis is an essential component of the minimum database.[3]

The Complete Urinalysis

A complete urinalysis combines evaluation of physical and chemical properties with microscopic evaluation of urine sediment. Physical characteristics are determined via visual and olfactory inspection and through the use of a refractometer, while urine chemistry is evaluated via multi-test dipstrips.[1,6]

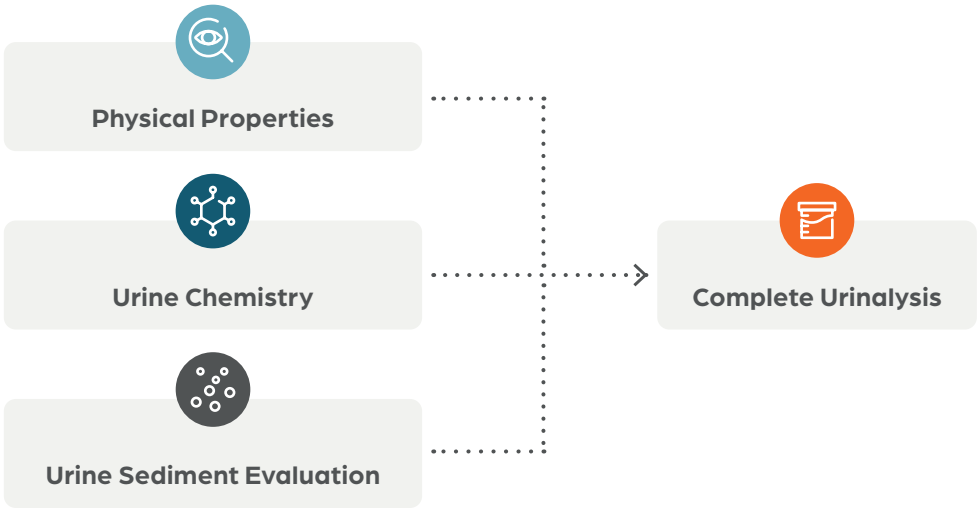


Figure 2: Three Broad Categories of Investigation Make Up a Complete Urinalysis

In addition to routine diagnostic health screenings, a urinalysis should be included in, but not limited to, the clinical scenarios seen in Figure 3 below[2]:

- Clinical signs of lower urinary tract disease
- Changes in character of urine
- Known, previous or suspected renal disease or urolithiasis
- Evaluation of animals with non-renal systemic disease, e.g. Hepatic Disease
- Evaluation of animals with suspected infectious disease
- In animals with fever
- To evaluate renal function in dehydrated animals prior to institution of fluid therapy
- As a screening tool for geriatric animals and pre-anaesthesia

Figure 3: Clinical Indications for a Complete Urinalysis (not exhaustive)

Collection Methods

Collection methods influence what is considered “normal” in urine sediment results. It is important to record collection method so the clinician can properly interpret results and then steer subsequent diagnostic and treatment decisions. Clinicians must consider collection method in their interpretation, as each method may artifactually increase certain elements in urine sediment findings (i.e. increased numbers of epithelial cells with catherisation, erythrocytes with cystocentesis, etc). Table 2 summarises the pros and cons of the three urine collection methods.[2]

Table 2: Urine Collection Methods – Benefits and Risks

Collection Method	 Voided Sample	 Catheterisation	 Cystocentesis
Benefits	– Generally low stress – Avoids trauma to urinary tract – Useful for initial routine evaluation of suspected urinary disorders and screening	– May avoid contamination from distal urogenital tract	– Avoid contamination – Best for culture – May be better tolerated and easier than Catheterisation especially cats
Risks	– Contamination from distal urinary tract with bacteria, cells, etc.	– Stress due to restraint and Catheterisation – Skill required – Potential trauma to tissues – Potential infection due to poor technique	– Potential tissue trauma – Stress due to restraint – Skill required

Urine Sample Processing

Urinalysis must be performed as quickly as possible after urine collection. The time of collection relative to the time of analysis is important because a delay can result in several changes to the urine (Figure 4). When these changes occur, the urinalysis results may change and no longer accurately reflect the patient.

Figure 4: Consequences of Urine Sample Processing Delays

Optimally, urine should be processed and examined within 15-60 minutes of collection. If the analysis cannot be performed promptly, the urine should be stored at a refrigerated temperature to minimise changes in urine physical and chemical makeup, inhibit bacterial growth and maximise cell preservation.[2,97]

Strict recommendations regarding the optimal duration of refrigerated storage cannot be made, because the unique urine components in each patient's sample determine the maximum acceptable duration of refrigerated storage. However, the most recent recommendation by experts is to perform the urinalysis, if possible, within 4 hours of refrigeration.[8] After refrigeration, it is extremely important to bring the sample back to room temperature prior to analysis, since refrigeration can cause in vitro formation of crystals. This crystal formation may inaccurately indicate the presence or extent of crystalluria *in vivo*.[9] If crystalluria is a clinical concern, freshly collected urine should be examined immediately.[10]

Because urinalysis results may be affected by storage duration and temperature, the time the urine was collected, the time it arrived in the clinic/laboratory, the time it was processed, and the method and length of storage should be recorded.

Traditional Manual Sediment Analysis

There is tremendous variability in the identification and quantification of formed elements in urine, due to a variety of factors. A 2009 study conducted by Wald et al., demonstrated that independent of years of experience of the observers, only "slight to moderate agreement" was seen when comparing microscopic evaluation results of urine sediment samples.[11]

Many issues outside the control of the clinician can also influence the accuracy of microscopic urine sediment analysis. The manual microscopic evaluation method for urine sediment traditionally requires review of at least 10 high-powered fields (hpf), the equivalent of 2µL of unspun urine, which may or may not represent all clinically significant elements. Counting cells in low concentrations results in high coefficients of variation (%CV), which increase mathematically when lower volumes of sample are examined. For example, 1µL of analyzed sample is associated with a 60% CV, but when analyzing 10µL of sample it drops to 18% CV.[12]

Traditional Manual Sediment Analysis (cont.)

The typical imprecision/inaccuracy observed in the manual method can easily affect clinical decisions. For example, with improper mixing of the sample, one aliquot may contain 1-3 RBC/HPF, while another aliquot prepared from the same sample may yield 10-15 RBC/HPF. This latter finding is abnormal and may trigger follow-up by the clinician.

If a sample urine volume is used that is different from the volume used to determine reference ranges, the reference ranges may no longer be appropriate. As outlined in Figure 6, the concentration of increasing volumes of urine could lead to increasing numbers of formed elements per HPF. In these cases, the deviation from the standardised quantity should be noted on the final report.

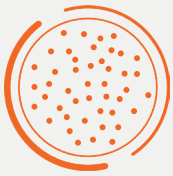
			
Starting Urine Volume	1.0 mL	2.0 mLs	3.0 mLs
Resuspension Volume	0.35 mL	0.35 mL	0.35 mL
WBC/HPF	15	30	45
Semi-Quantitative Bucket	6 – 20 WBC/HPF	21 – 50 WBC/HPF	21 – 50 WBC/HPF

Figure 6: Urine Sediment Formed Element Quantification

The use of stain, or lack thereof, can also impact the imprecision/inaccuracy of visual methods. When stain is not used, it can be difficult to discriminate between cells that have similar appearances. On the other hand, non-specific stains can colour the background and may obscure some formed elements such as RBCs or introduce artefactual changes to the sample.[12]

Unstained urine sediment is examined under reduced illumination by either lowering the microscope condenser and/or closing the substage iris diaphragm. The lower condenser position provides the necessary contrast to identify formed elements in the urine. The sample must be evaluated in its entirety under multiple magnifications to allow for visualisation of all formed elements.[13] This technique requires skill and can be time consuming. The technical expertise and time needed to perform a consistent urine sediment microscopic analysis are notable barriers to in-hospital examination of fresh urine.[5] Automating this process can alleviate these barriers.

Achieving Consistency in Urine Sediment Analysis[6,8]

As previously demonstrated, a high coefficient of variation can exist in manual urine sediment analysis due to a large number of pre-analytical and analytical factors in sample handling and microscopic evaluation.[12] With the Vetscan Imagyst AI Urine Sediment sample preparation method and artificial intelligence (AI) algorithm, consistency of results can be achieved by any hospital team member. With this innovative process, there is a standardised starting volume, residual volume, volume of sample placed on the slide, as well as centrifugation time and relative centrifugal force (RCF). Moreover, the AI algorithm provides consistent, precise and accurate results at all times of the day, eliminating variation between microscopic readers.[16] Vetscan Imagyst standardises these factors for your practice.

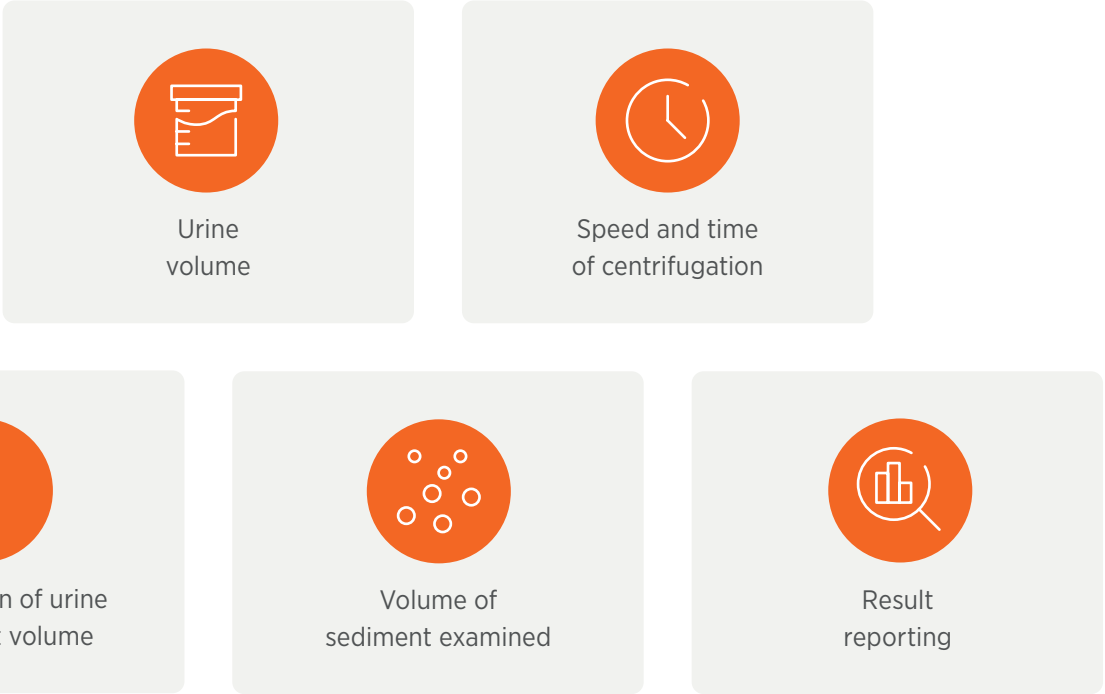


Figure 7: standardisation of the Urinalysis by Vetscan Imagyst AI Urine Sediment

Image Recognition AI: Deep Learning versus Superficial Learning

Not all image recognition AI is the same. The methods currently used in the veterinary market to train models and classify data are known as Superficial and Deep Learning. Two key differences between superficial and deep learning algorithms are the way in which the algorithm is trained, and the number of "features" used in the classification of new objects. "Features" are distinguishing characteristics that visually make an object that object (e.g., a cat has whiskers, fur, two eyes, four legs, etc.). With superficial learning AI algorithms, a human manually selects the "features" of an object, and the algorithm uses these features to classify new objects.

Image Recognition AI: Deep Learning versus Superficial Learning (cont.)

In deep learning AI algorithms, object "feature" selection and training are not limited by manual or human selection. This step is skipped. In deep learning, countless numbers of objects or data are shown to the algorithm and the algorithm extracts relevant "features" that it uses to classify new objects. During the training process, an expert tells the algorithm whether it has classified objects correctly, allowing the AI to learn and extract more features with each correction. These processes are illustrated in Figure 8 below.

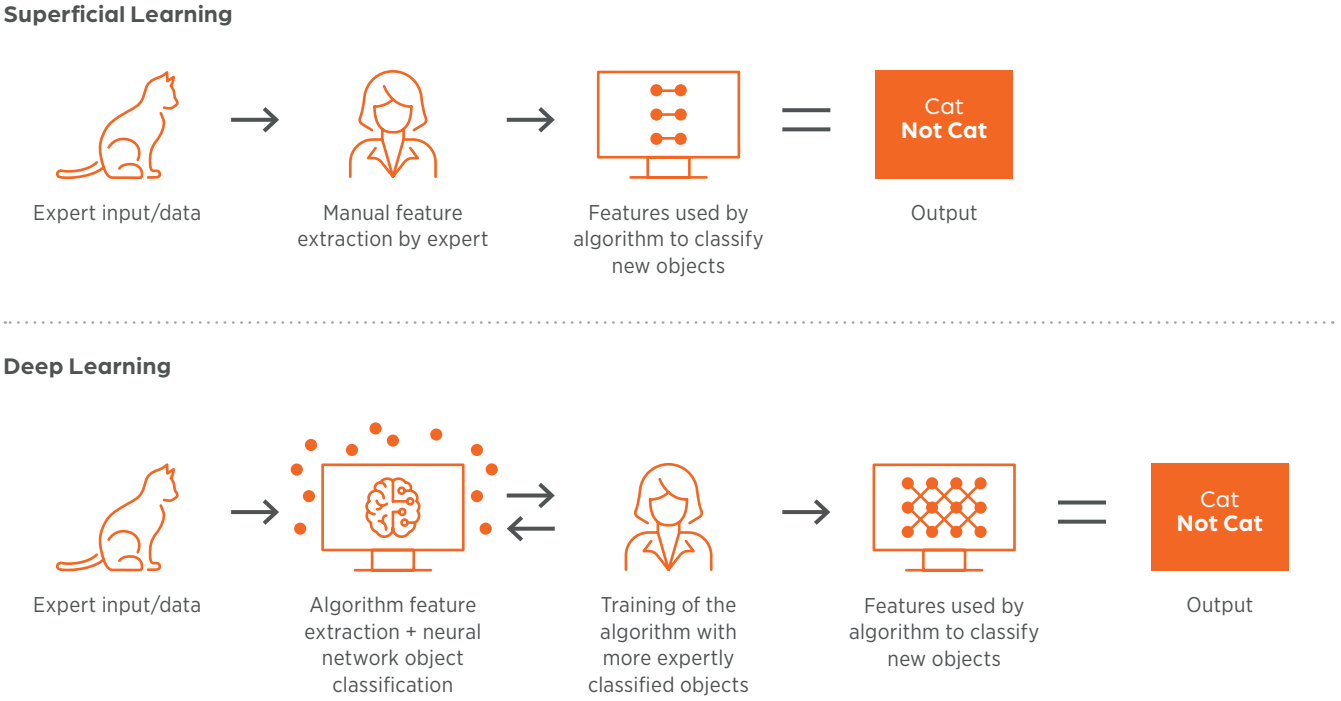


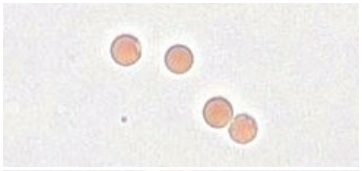
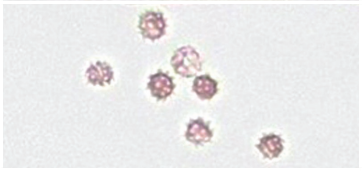
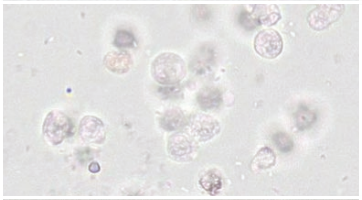
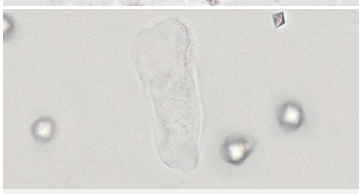
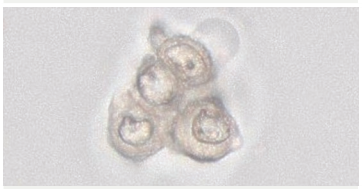
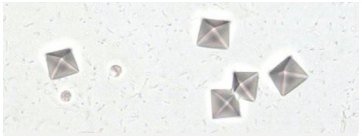
Figure 8: Types of Image Analysis AI Algorithms

In superficial learning fewer features are evaluated, because it relies on a human expert to identify every feature used to train the algorithm (i.e. the human tells the algorithm what attributes of a cell to pick out). It can be difficult to improve these algorithms as the human needs to visualise more features to teach the algorithm.[17] On the other hand, deep learning AI systems use thousands of features and their inter-relations to identify an object. The algorithm determines differentiating attributes that we often cannot cannot pick up with our eyes (i.e. the human expert tells the algorithm, "this is a cell" and the algorithm breaks the image down to the pixel level to decide what key attributes define a "cell"). Improvements to deep learning algorithms are generally related to providing more and better image examples so the combined feature extraction and analysis components of deep learning can be maximised.[15]

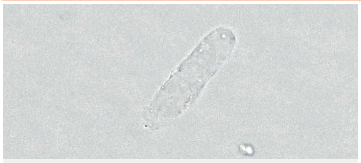
The deep learning system that is utilised for the Vetscan Imagyst is a convolutional deep neural network. A convolutional deep neural network uses a very large number of small filters to extract a very large number of features from the image which are applied to the deep learning neural network. Deep learning AI will excel in identification of often difficult to visualise urine sediment elements compared to superficial learning AI.

The Vetscan Imagyst AI Urine Sediment algorithm detects and reports semi-quantitatively the elements outlined in Table 3.

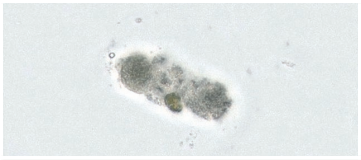
Table 3: Urine Sediment Elements

Urine Sediment Elements[8]	
 	**Red Blood Cells** The most common causes of haematuria in veterinary medicine are bacterial infections, neoplasia and trauma (cystocentesis, urolithiasis, injury). The causes of haematuria can be grouped in categories such as renal parenchymal disease, renal vascular disease, lower urinary tract disorders (including trauma), and systemic coagulation disorders.[8] Crenated RBCs can be seen in highly concentrated urine samples, particularly those with delayed processing. The change in cell morphology is the result of cell dehydration.
	**White Blood Cells** The most common causes of pyuria in veterinary medicine include infection-upper or lower urinary tract, urolithiasis, neoplasia and inflammation or infection of the genital tract.[8]
 Squamous  Other Epithelial	**Squamous, Transitional (Urothelial) and Renal Tubular Epithelial Cells** Increased numbers of squamous epithelial cells most commonly occur due to oestrus, neoplasia and collection of urine via catherisation. Small numbers are also common with voided samples as a result of normal cell turnover in the urinary tract. While small numbers of transitional (urothelial) cells may also be observed in urine due to normal cell turnover, the presence of renal tubular cells always indicates pathology. Clumping of epithelial cells is also considered abnormal. If clumping, abnormal cell morphology or increased numbers of epithelial cells are observed, consider investigation of infection, neoplasia, urolithiasis, AKI, or sterile inflammation (feline idiopathic cystitis).[8] Submitting a stained urine sediment smear for Add-On Expert Review* is recommended.
 Struvite  Calcium Oxalate Dihydrate	**Struvite and Calcium Oxalate Dihydrate Crystals** Struvite and calcium oxalate dihydrate crystals may be found in normal dogs and cats, and do not guarantee uroliths are present. Based on symptoms, investigation may be warranted to rule out UTI or stone formation.[8]

Urine Sediment Elements[8] (cont.)



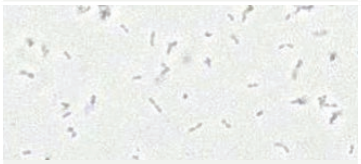
Hyaline



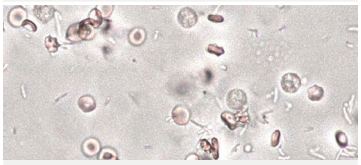
Non–hyaline

Hyaline and Non–hyaline Casts

Increased numbers of non-hyaline casts usually indicate the presence of renal pathology. Consider Add-On Expert Review* for further evaluation. Increased numbers of hyaline casts may be seen with increased urinary protein due to pre-renal or renal causes of proteinuria.



Cocci



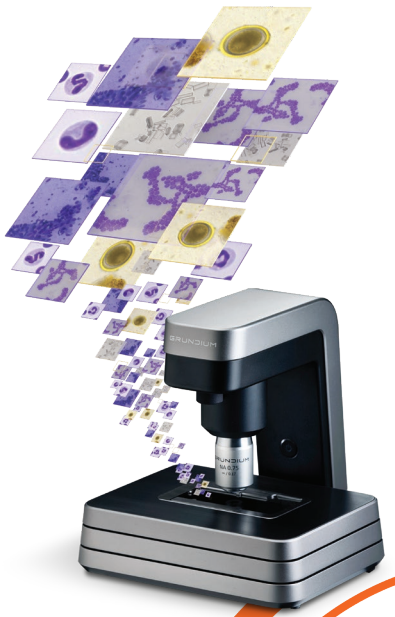
Rod

Cocci and Rod Bacteria

Interpret significance considering clinical signs, presence or absence of WBC and collection method. For more information, consider an Add-On Expert Review* with a stained sediment smear. To guide antimicrobial selection and/or confirm suspected bacterial infection, perform a culture and sensitivity.

The Vetscan Imagyst® AI Urine Sediment application can alleviate the challenges of in-hospital urine sediment examination. The system streamlines workflow and standardises urine sediment processing and evaluation. It provides a succinct report, including pictures, which can be shared with pet owners.

These detailed reports may increase owner perception of the value of urine sediment examination and drive compliance with prescribed treatment. Further, the veterinary practitioner can have confidence in the accuracy of results for urine sediment samples and easily request further diagnostic information with an Add-On Expert Review* by a clinical pathologist.[14]



References

1. Barsanti, Jeanne A. Small Animal Clinical Diagnosis by Laboratory Methods, 5th edition, Elsevier 2012: Chapter 7: Urinary Disorders. Pp. 131-143, 152.
2. Chew, Dennis and DiBartola, Stephen. Interpretation of Canine and Feline Urinalysis. Nestle Purina, Wilmington, DE. 2004: pp.1-31
3. Stockham, Steven L. and Scott, Michael A. Fundamentals of Veterinary Clinical Pathology, Second edition, Blackwell Publishing 2008: Chapter 8: Urinary System. Pp.8, 436-480
4. Bottini PV, Martinez MH, Garlipp CR. Urinalysis: comparison between microscopic analysis and a new automated microscopy image-based urine sediment instrument. Clinical Laboratory Lab. 2014; 60(4):693-697.
5. Gibbs, N.H. et al. Use of urinalysis during baseline diagnostics in dogs and cats: an open survey. Journal of Small Animal Practice: 2023. pp. 64, 88-95.
6. Skeldon, N., and Ristić, J. (2016). BSAVA Manual of Canine and Feline Clinical Pathology (Vol. Third edition). Quedgeley, Gloucester: BSAVA. P 183-205.
7. Chew, Dennis and Schenck, Patricia A. Urinalysis in the Dog and Cat. First edition. Wiley Blackwell. 2023: pp. 162
8. Chew, Dennis and Schenck, Patricia A. Urinalysis in the Dog and Cat. First edition. Wiley Blackwell. 2023: pp. 177-216.
9. Albasan H, Lulich JP, Osborne CA, Lekcharoensuk C, Ulrich LK, Carpenter KA. Effects of storage time and temperature on pH, specific gravity, and crystal formation in urine samples from dogs and cats. J Am Vet Med Assoc. 2003 Jan 15;222(2):176-179.
10. Sturgess, CP, Hesford A, Owen H and Privett R. An investigation into the effects of storage on the diagnosis of crystalluria in cats. J Fel Med Surg 2001;3:81-85.
11. Wald, R. et al. Interobserver Reliability of Urine Sediment Interpretation. Clin J Am Soc Nephrol 2009; 4: 567-571.
12. Kouri T, Gyory A, Rowan RM. ISLH Recommended Reference Procedure for the Enumeration of Particles in Urine. Laboratory Hematology 2003; 9:58-63.
13. Morissette, E. Evaluation of Urine by Automatic and Manual Methods. ABX-00145.
14. Zoetis data on file. Study No. DHXMZ-US-23-218 (DX218)
15. https://www.ibm.com/blog/ai-vs-machine-learning-vs-deep-learning-vs-neural-networks/ Accessed 08/28/23

* Additional costs apply for Add-On Expert Review

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