## One Step



# VET-10

### **REAGENT STRIPS FOR VETENIARY URINALYSIS INTENDED USE**

Urinalysis is an important tool in disease detection, as well as monitoring and screening animal health. Abnormalities can be indicative of diseases of the urinary system as well as other organ systems, including liver function, acid-base status, and carbohydrate metabolism. Complete urinalysis involves both macroscopic and microscopic assessment. This is typically performed by gross visual assessment of the urine, microscopic examination, and chemical evaluation. Several chemical parameters can be measured using a commercially available in-house dipstick test. This test is relatively inexpensive and takes less than 2 minutes to complete. Typical dipstick strips include the following tests: bilirubin, blood, glucose, ketones, pH, protein, specific gravity, urobilinogen, leukocytes, and nitrite

### STORAGE AND HANDLING

Store in a cool, dry place at temperatures between 2°C ~30 °C. Do not store the strips in a refrigerator or freezer. Store away from moisture and light. When stored in the original bottle or pouch, the product is stable up to the expiry date printed on the label and (or) vial box.

\*Note: When using the bottle packaged product, please replace the bottle cap immediately and tightly after removing test strips, and keep the bottle tightly closed between tests.

\*Note: When using the pouched product immediately reseal the foil pouch after removing the test strip.  $\ensuremath{\mathsf{Do}}$ not remove desiccant from bottle or pouch. Do not touch test areas of urine reagent strips. Do not open the container until it is ready to use. Discoloration or darkening of the test pads may indicate deterioration. If this is evident, or if test results are questionable or inconsistent with expected finding, confirm that the product is within its expiration date and is reacting properly using known negative and positive control materials. Do not use after the expiry date.

\*Note: Once the bottle has been opened, the remaining strips remain stable for up to 6 months. \*Note: Once the pouch has been opened, the remaining

strips remain stable for up to 30 days.

### QUALITY CONTROL

For best results, performance of reagent strips should be For best results, performance or reagent strips should be confirmed by testing known negative and positive specimen or controls (e.g., **Quantimetrix** Dipper Urine Dipstick, Dropper Urine Dipstick, Dip&Spin Urine Dipstick; **Bio-Rad** qUAntify Plus Control; **Thermo SCIENTIFIC** MAS UA Control) whenever a new bottle is first opened. Each laboratory should establish its own goals for adequate standards of performance. Each lab worker should ensure that it complies with government and local requirements.

#### SAMPLE COLLECTION

Urine should be collected in a clean, dry container that is free of any disinfecting or cleaning chemicals. Samples may be collected by free catch of voided sample, manual bladder expression, catheterization, or cystocentesis Voided samples are the easiest and least invasive samples

collect. However, voided samples may have to contaminants that include bacteria, epithelial cells, and white blood cells.<sup>1</sup> Red blood cells should not be found in normal voided samples. Voided samples should be collected midstream to lessen contaminants from the vagina or prepuce. Collection of samples from surfaces such as floors, cages, and litter boxes should be avoided, since these will introduce environmental contaminants.

Manual expression of the bladder is another technique used in urine collection. In this method, the patient's bladder is gently squeezed until urine is expressed. This technique may lead to bladder trauma resulting in hematuria, and in some instances (such as urethral obstruction) may result in a ruptured bladder. This method may have the same cellular contaminants as a voided sample

Catheterization is performed by placing a small hollow tube into the urethra to the level of the bladder. Urine is then withdrawn from the bladder using a syringe. Catheterized samples have less contamination from the distal urogenital tract; however, contamination from the urethra may still occur. Contaminants include epithelial cells or red blood cells. Poor catheterization technique may lead to trauma or, less commonly, infection.

Cystocentesis samples are collected by inserting a sterile needle through the body wall into the bladder. Urine is withdrawn from the bladder using a syringe. A lateral or ventral approach to the bladder may be made without causing severe trauma to any vital region of the bladder. Clipping or surgical preparation of the area along the body wall is not necessary prior to sample collection. Often a inch or 1.5-inch 22-gauge needle is used attached to a 6 or 12 cc syringe. The bladder is manually immobilized, and the needle is inserted through the abdominal wall into the bladder, and the urine is withdrawn. It is important to stop aspirating prior to withdrawing the needle as this may lead to aspiration of blood cells or epithelium from the bladder wall. Animals often tolerate cystocentesis very well and little restraint is needed.

Contaminants that may be found include iatrogenically introduced red blood cells. Rarely, enterocentesis may occur which results in a sample containing bacteria. intestinal villi and other intestinal contents.

### SAMPLE HANDLING

To obtain accurate results, the urine collection, storage, and handling must be sterile and follow standard procedures. The dipstick analysis should be performed as soon after collection as possible (ideally within 30 minutes of collection) and the sample should be well mixed prior to testing. If for some reason the test cannot be performed immediately, the sample may be covered and refrigerated. It should be allowed to return to room temperature prior to testing. The dipsticks should be stored in the original airtight container to maintain reagent reactivity

### TESTING METHODS

Dipsticks may be removed from the airtight, light sealed containers. It is important not to touch the reagent areas of the strip as this may alter test results. Each reagent area should be immersed in urine by dipping. The excess urine should be removed to prevent dilution of reagents or mixing of reagents between pads. This can be achieved by tilting the strip and allowing the urine to run off the edges. While blotting excess urine, ensure the chemicals from the different tests do not mix.

The reagent pads should be read at specified times. These times are different for each test and vary between dipstick manufacturers. Compare the blocks to the corresponding color chart provided by the test strip's manufacturer.

Urine discoloration may create difficulty in visually interpreting the test results. Color changes may be masked or read as false positive test results. If the urine is noticeably discolored, the sample may be centrifuged, and the supernatant used for analysis.

### VISUAL TEST PROCEDURE

The procedure must be followed exactly to achieve reliable results. Do not compare strips with color chart before the strip is dipped in urine

1) Dip the strip into the urine up to the test area for no more than two seconds. 2) Draw the edge of the strip along the brim of the vessel to remove excess urine, but make sure the reagent pads do not meet the brim of the vessel.

Turn the strip on its side and tap once on a piece of absorbent material to remove any remaining urine; excessive urine on the strip may cause the interaction of

chemicals between adjacent reagent pads, so that an incorrect result may occur. 3) Compare the colors of the reagent pads exactly after 60 seconds (Leukocytes after 90~120 seconds) with the color chart on the vial label under good light. While comparing, keep the strip horizontal to prevent possible mixing of chemicals when excessive urine is present

chemicals when excessive urine is present

### UROBILINOGEN

### Read the test result at 60 seconds.

Urobilinogen is formed by intestinal bacteria from the breakdown of conjugated bilirubin. Urobilinogen is usually excreted in feces, however a small amount may be reabsorbed and excreted in urine. Limited diagnostic value in veterinary medicine.

The dipstick method measures urobilinogen by reacting with p-diethylaminobenzaldehyde in an acid environment. A positive test response indicates normal enterohepatic circulation of biliary pigments. High concentrations of biliary pigments may occur in hemolytic crisis, or cases of hepatic or intestinal dysfunction.

False Negatives

In stored urine because urobilinogen is unstable when exposed to light and air. **Clinical Significance** 

Cholestatic hepatitis biliary obstruction. False Positives

Administration of certain drugs

### GLUCOSE

Read the test result at 60 seconds. Glucose is not detectable in the urine of healthy dogs or cats. In a healthy animal, glucose passes freely through the glomerular filter and is resorbed by the proximal tubules. If glucosuria is present, it is due to either an excess amount of glucose reaching the tubules that cannot be resorbed or, less commonly, decreased tubular resorptive function.

Reagent strips measure glucose levels using the glucose oxidase method. This method is a sequential enzymatic reaction. Glucose reacts with glucose oxidase to produce hydrogen peroxide, which oxidizes the indicator chemical to produce a color change. The color change is related to the amount of glucose present in the urine sample. Glucosuria may be either persistent or transient and multiple tests may be needed for differentiation of these conditions. Persistent causes of glucosuria include diabetes mellitus administration of glucose containing fluids, chronic disease that is not related to the kidneys such as hyperadrenocorticism, hyperpituitarism, or acromegaly. Other diseases that may result in transient hyperglycemis leading to glucosuria include hyperthyroidism, acute pancreatitis, stress (especially in cats), postprandial, and administration of certain drugs. Rarely, a Fanconi-like syndrome may lead to glucosuria. False Negatives

### Cold urine / High concentrations of vitamin C or ketones

High urine specific gravity (values lower than normal may occur)

### False Positives

Contact with bleach or hydrogen peroxide. Treatment with corticosteroids, aspirin, ephedrine, morphine, furosemide, dextrothyroxine, anesthesia Clinical Significance

Diabetes mellitus

Renal tubular disease and non-renal chronic disease in cats both may cause glycosuria without hyperglycemia. /Administration of glucose containing fluids. Hyperadrenocorticism /Endocrine disorders /Pancreatitis /Stress in cats

### BILIRUBIN

### Read the test result at 60 seconds.

Bilirubin is produced from the breakdown of hemoglobin, transported to the liver bound to albumin, and conjugated with carbohydrates by hepatocytes. Only conjugated bilirubin is found in urine. Excess bilirubin may be produced when red blood cells are destroyed, or in liver disease, including bile duct obstruction. Conjugated bilirubin is detected in urine if the renal threshold is exceeded. The renal threshold in dogs, especially males, is lower than that of other species. Reagent strips measure levels of conjugated bilirubin with the diazotization method. This occurs by coupling bilirubin with diazotized dichloroaniline in an acidic environment. Bilirubin is very unstable when exposed to room air and light. Thus, urine specimens should be tested soon after collection. Positive test results may be observed in concentrated urine of healthy dogs. In dogs, the renal threshold for bilirubin is low and renal tubules can break down heme and produce some renal bilirubin, therefore slight bilirubinuria can be a normal finding in dogs with concentrated urine. However, bilirubinuria is always abnormal in cats. Bilirubinuria may indicate liver disease, bile duct obstruction, starvation, hemolysis, or pyrexia. Bilirubinuria in bile duct obstruction is often more severe than that of hepatocellular disease. False Negatives

Samples with large amounts of vitamin C False Positives

High concentrated urine in dogs

High doses of chlorpromazine and phenazopyridine **Clinical Significance** 

Hemolyticanemia /Liver disease /Bile duct obstruction/Prolonged fasting/Fever

The detection of bilirubin in urine may precede clinically

evident jaundice, so that it may be an early indicator of disease

In dogs, traces of bilirubin in concentrated urine may be physiological.

In cats, bilirubin in urine is always abnormal.

### **KETONES**

Read the test result at 60 seconds. Acetone, acetoacetic acid, and beta-hydroxybutyric acid are ketones. Glomeruli freely filter ketones, and the tubules then resorb them completely. If the tubular resorptive capacity is saturated, then the ketones are incompletely resorbed, resulting in ketonuria. Ketonuria occurs quickly in hne معeil ketonemia. Ketonuria does not signify renal disease, but rather excessive lipid or defective carbohydrate metabolism. Dipstick tests are semiquantitative and only detect acetone and acetoacetic acid. Reagent strips contain nitroprusside that does not react with beta-hydroxybutyric acid.

gravity of the latter is expected to be low. Low values may be normal in overhydrated animals. High values may be normal in dehydrated dogs Low urine specific gravity values observed in successive samples are indicative of renal impairment caused by diabetes insipidus, hypocalcemia, liver disease and psychogenic polydipsia.Oliguria + low urine specific gravity = acute renal failure For greater clinical significance it is

### BLOOD

Read the test result at 60 seconds. The occult blood test will react positively in the presence of red blood cells, free hemoglobin, or free myoglobin. Hemoglobin usually is bound and is too large to pass through the glomerular filter. If the renal threshold is exceeded, the hemoglobin can pass into the urine Mvoalobin r har passes through the glomerular filter. Myoglobin can be detected in urine before a change in plasma color is apparent. Healthy animals should have negative test results. This test is based on a pseudoperoxidase reaction, which is more sensitive to hemoglobin and myoglobin than intact red blood cells.A positive occult blood test indicates hematuria hemoglobinuria, or myoglobinuria. Hematuria can be caused by trauma, infection, inflammation, infarction, calculi, neoplasia, or coagulopathy anywhere along the urinary tract. In cases of hematuria, the urine is red and cloudy, but will clear if centrifuged Microscopic evaluation of the urine sediment will reveal red blood cells. Hemoglobinuria, on the other hand, will have reddish brown urine that does not become clear after centrifugation. The microscopic evaluation of urine sediment will not reveal red blood cells. With intravascular hemolysis, plasma will have a reddish tint due to hemoglobinemia that is detectable prior to hemoglobinuria. The patient usually will be clinically anemic.

Contamination of the sample with bleach, iodine, or bromine Heat in bitches./Urine was obtained by catheterization.

### Read the test result at 60 seconds.

The pH of urine can vary depending on an animal's diet as well as its acid-base status. For example, animals that primarily eat high protein meat-based diets will have acidic urine. On the other hand, animals that eat more vegetablebased diets will have alkaline urine. The urine sample should be fresh as urine becomes more alkaline on standing due to the conversion of urea to ammonia by bacteria (if present), and loss of  $CO_2$ . Clinical significance of acidic urine

Animals on high protein diets /Metabolic acidosis

Treatment with acidifying agents/Hypochloremia Acidic urine: oxalate stones, uric acid and cystine I values cats and

interpreted based on the clinical history, urine collection method, density, and sediment.

Calculate the protein/creatine ratio (UPC) to obtain a more reliable indicator for kidney damage, as this ratio is used to correct the variation resulting from urine volume

### **False Positives**

Hematuria or pyuria caused by infection or inflammatory conditions are often associated with proteinuria./Highly alkaline urine

Urine contaminated by benzalkonium chloride disinfectants. Urine with urease-producing bacteria

Treatment with ampicillin, barbiturates, aspirin, bacitracin, steroids gentamicin, kanamycin, phenylbutazone. streptomycin, sulphonamides

### NITRITE

Read the test result at 60 seconds.

animals which produce large volumes of urine, specific

recommended to use first morning urine.

### **False Negative**

Uncommon in dogs in fresh urine. Very common in stored urine as ketones are highly volatile. Common in cats because it doesn't detect B-Hydroxybutyric acid which is the main ketone in cats.

#### False Positives

Very common caused by highly pigmented urine or high concentrations of levodopa metabolites in urine. **Clinical Significance** 

Increased lipid catabolism (starvation, diets low in carbohydrates and high fat) Persistent hypo-glycaemia (insulinoma) /Diabetic ketoacidosis/Fever/ Intense exercise

### SPECIFIC GRAVITY

### Read the test result at 60 seconds.

Urine specific gravity is based on the ratio of weight of urine to weight of an equivalent volume of pure water. This test is used to measure tubular function. The dipstick measures specific gravity by measuring the change in pKa of polyelectrolytes in relation to ionic concentration. High lipid content in urine may also alter the results by either raising or lowering the specific gravity measurement. False Negatives

### Low reading may occur if the urine is alkaline

### False Positives

Falsely elevated by moderate to high concentrations of protein (Proteinuria)

### **Clinical Significance**

Measures the renal tubular concentrating ability or how dilute the urine is. Strips provide approximate density readings, so that their diagnosis value is merely indicative. The use of a refractometer is recommended for greater reliability

Normal circumstances (1001-1065 in dogs, and 1001-1080 in cats).

Heavily influenced by diet, hydration, and urine volume. In

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A false positive test result may occur if the urine is contaminated with bleach or contains large amounts of iodide or bromide.

Further evaluation of the urine sediment is needed if a positive test result is found. Most commonly, hematuria is the cause of the positive test result while myoglobinuria is rare

#### **False Negatives**

Urine samples not homogenized before testing (erythrocytes sediment rapidly) formalin used as a preservative.

#### Presence of nitrates

#### **Clinical Significance**

Hematuria due to trauma, infection, inflammation, kidney stones, neoplasms

Hemoglobinuria/Myoglobinuria/Sample obtained catheterization.

Contamination of genital tract due to prostatic, uterine, vaginal, or preputial disease /Idiopathic cystitis in case/Parasites (Capillaria plica) Treatment with cyclophosphamide False Positives

### Clinical significance of alkaline urine

Animals on a grain-rich diets /Metabolic alkalosis/Treatment with alkalizing agents/Urinary tract infection by proteus and staphylococcus

Alkaline urine; struvite, calcium carbonate, calcium phosphate, ammonium, and phosphor crystal stones.

### PROTEIN

### Read the test result at 60 seconds.

Dogs and cats normally have small proteins that pass through the glomerular filter; however, most of these proteins are resorbed by the renal tubules. The renal nephron does excrete a small amount of Tamm Horsfall protein. Thus, only a very small amount of protein is normally excreted in the urine, which is not usually clinically detectable

The protein portion of the dipstick reagent strip measures the protein based on a pH dye indicator method using bromophenol blue. Proteinuria may be due to hemorrhage infection, intravascular hemolysis, or renal disease. Hemorrhage is confirmed by a positive occult blood reaction on the dipstick and the presence of red blood cells in the sediment. A urinary infection or cystitis can be confirmed by observing bacteria and white blood cells on sediment examination. Cases of intravascular hemolysis have hemoglobinuria leading to a positive occult blood test. Proteinuria of renal disease may be due to glomerular and/or tubular lesions. If the proteinuria is due to renal disease, the occult blood test will be negative, and the sediment may or may not contain casts.

If the urine protein dipstick is positive for protein, the sample should be further analyzed with a quantitative method at an outside laboratory.

### False Negatives

### Acidic or highly diluted urine

Proteinuria is due to proteins other than albumin, such as globulins or Bence-Jones proteins to which the reagent on the strip is less sensitive.

### Clinical Significance

Glomerular disease /Glomerulonephritis Amyloidosis Highly dependent on specific gravity. The same protein levels in urine may be abnormal in diluted urine, or physiological in concentrated urine. The results should be

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The nitrite portion of the dipstick analysis has limited value in veterinary medicine.

Negative test results do not exclude infection. The urinary tract infection may involve organisms that do not convert nitrites, or the urine may not have been held in the bladder greater than 4 hours.

### **Clinical Significance**

Infection with Gam+ bacteria

For more accurate results the urine should spend at least 4 hours in the bladder. Therefore, it is best to collect a (first) morning sample or ensure the patient has not urinated in at least 4 hours

### False Positives

Very dark urine/Very high specific gravity urine Large quantities of vitamin C in the urine sample

### LEUKOCYTES

Read the test result between 90~120 seconds. False Negatives

Very high specific gravity/Glycosuria Treatment with high doses of cephalexin, tetracycline, or other antibiotics

### **Clinical Significance**

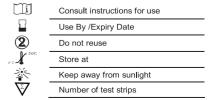
Dogs: pyuria inflammation or urinary tract infection (a negative result does rule out infection) Cats: no clinical significance due to the high number of

false positives

### False Positives

Fecal contamination Samples obtained by urination in dogs with prostatitis or pyometra.

#### NOTES ON SYMBOLS



### DFI CO., Ltd

388-25, Gomo-ro, Jillye-myeon, Gimhae-si, Gyeongsangnam-do, 50875 Republic of Korea