

One + Step[®] VET-10

REAGENT STRIPS FOR VETERINARY 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, nitrite analyses.

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 container, the product is stable up to the expiry date printed on the label and (or) vial box. Replace the bottle cap immediately and tightly after removing test strips, and keep the vial tightly closed between tests. Do not remove desiccant from bottle. Do not touch test areas of urine reagent strips. Do not open container until 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 canister has been opened, the remaining strips remain stable for up to 6 months.

QUALITY CONTROL

For best results, performance of 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 to collect. However, voided samples may have contaminants that include bacteria, epithelial cells, and white blood cells.¹ 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 1 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

In order 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 air tight, 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 the specified times. These times are different for each test and also 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.

Urobilinogen

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. This test is not of significant value in animals.

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.

A false negative test result may occur if there is formalin residue in the collection container, or if the sample is old, because urobilinogen is very unstable when exposed to light and air.

Glucose

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 hyperglycemias 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 positive test results may be caused by contamination of the sample with oxidants such as hydrogen peroxide, bleach (sodium hypochlorite), or occasionally pseudoglucose in obstructed cats.

False negative test results may be due to high concentrations of ascorbic acid (Vitamin C) in the urine. Moderately high concentrations of ketones also may cause false negative test results if the amount of glucose is only slightly elevated. The glucose test also becomes less reactive as urine specific gravity increases or as temperature decreases. Cold urine (refrigerated specimens) or expired reagent strips may also result in false negative test results.

Bilirubin

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 are able to 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 positive test results may occur if high doses of chlorpromazine, which lowers urine pH, have been given. A metabolite of etodolac (Lodine), also produces false positive test results. False negative test results may occur in urine samples with high ascorbic acid or nitrite concentration.

Ketones

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 younger animals and is more easily detected than 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. Ketonuria may be caused by starvation, insulinoma, diabetic ketoacidosis, persistent hypoglycemia, high fat low carbohydrate diets, and glycogen storage disease.

False positive test results may occur if urine is pigmented, or has high concentrations of levodopa metabolites.

Specific Gravity

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.

Although dipstick strips do have a method of approximating specific gravity, this measurement is best made with a refractometer.

Urine specific gravity measured by the dipstick can be falsely elevated by moderate to high concentrations of protein. Low reading may occur if the urine is alkaline. High lipid content in urine may also alter the results by either raising or lowering the specific gravity measurement.

Blood

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. Myoglobin on the other hand, is not bound and freely passes through the glomerular filter. Myoglobin can be detected in urine before a change in plasma color is apparent. The presence of free red blood cells results in a positive test when blood cells lyse and hemoglobin is released. 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. 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.

Hematuria can be caused by trauma, infection, inflammation, infarction, calculi, neoplasia or a 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.

A false positive test result may occur if the urine is contaminated with bleach, or contains large amounts of iodide or bromide. If a voided sample is collected from a bitch in heat, a false positive test may also occur. In this case a cystocentesis sample is preferred for analysis. Microbial peroxidase that is present in some urinary tract infections, can also lead to false positive test results. False negative test results may occur if the urine is not well mixed prior to evaluation. This is due to the fact that red blood cells often sediment quickly.

pH

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 vegetable-based diets will have an 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 CO₂. Causes of acidic urine include: meat diet, systemic acidosis, hypochloremia, and administration of acidifying agents such as D,L-methionine or NH₄Cl. Urine with high concentrations of glucose may have a lower pH. This is due to bacterial metabolism of glucose and and production of ammonia which lowers pH.

Causes of alkaline urine include: vegetable based diet, bacterial infection of urease-producing bacteria, systemic alkalosis, urine exposed to room air for an extended time (loss of CO₂), and administration of alkalinizing agents including citrate or NaHCO₃.

Urine pH also may provide good predictive assessment of crystal and stone morphology as certain crystals and stones form in either acidic or alkaline environments. Uric acid, cystine,

and calcium oxalate crystals are found in acidic urine. On the other hand, struvite, calcium carbonate, calcium phosphate, ammonium biurate, and amorphous phosphate crystals are found in alkaline urine.

For a more accurate assessment of urine pH, a pH meter may be used. However, for most routine veterinary analyses a dipstick pH reading is sufficient.

Protein

Dogs and cats normally have small proteins that pass through the glomerular filter, however a majority 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 bromphenol blue. Due to the negative charge of albumin, if protein (albumin) is present in urine, the pH increases, and a positive test result occurs. This test is primarily sensitive to albumin is relatively insensitive for the detection of globulins and Bence-Jones proteins. Positive protein results must be evaluated in relationship to the patient's history, physical examination, method of urine collection, urine specific gravity, and microscopic sediment examination. 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. Determination of the urine protein/urine creatinine ratio is helpful in confirming renal proteinuria.

Protein results must be analyzed with the urine specific gravity. Trace proteinuria may represent significant protein loss with low specific gravity, but not with high specific gravity. False positive protein reactions may occur with alkaline urine or if a disinfectant residue is in the urine, possibly from improper cleaning of the collection container. Samples containing urease-producing bacteria may have an elevated pH resulting in a false positive test result.

False negative test results may occur in dilute or acidic urine.

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

Nitrite

The nitrite portion of the dipstick analysis has limited value in veterinary medicine. This is due to the high number of false negative test results in small animals. Nitrites occur in urine during some bacterial infections. In order to achieve an accurate positive test result, the urine must have been retained in the bladder at least 4 hours. Therefore, it is best to collect a (first) morning sample or ensure the patient has not urinated in at least 4 hours.

A positive test indicates a bacterial infection. Gram negative rods are more likely produce a positive test response.

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.

Leukocytes

The leukocyte test detects the presence of white blood cells or partial cells in the urine. In dogs, this test is indicative of pyuria but false negative test results often occur.

False positive test results often occur in cats, and this test is clinically unreliable. False positive test results also may occur in the event of fecal contamination.

False negative test results may develop if the patient has been treated with high doses of tetracycline or other antibiotics. Glucosuria or increased urine specific gravity may cause false negative test results. False negative test results may be observed with voided urine samples obtained from animals with pyometra or prostatitis.

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NOTES ON SYMBOLS



Consult instructions for use



Use By /Expiry Date



Do not reuse



Store at



Keep away from sunlight



Number of test strips