



## Tagesu Abdisa Serbessa\*

Jimma University, School of Veterinary Medicine,  
Jimma, Oromia, Ethiopia

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\*Corresponding author: Tagesu Abdisa Serbessa,  
Jimma University, School of Veterinary Medicine,  
Jimma, Oromia, Ethiopia, Tel: +251933681407,  
E-mail: [abdisatagesu@gmail.com](mailto:abdisatagesu@gmail.com)

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## Research Article

# Examination of urine

Urinalysis usually is performed as a screening technique for acquiring a large amount of information about several body systems. It is studying the kidneys related to the detection of abnormal component in urine that may be of renal origins and relation of urine concentration ability to kidney function. Analysis of urine is conducted through collection of samples. Urine is normally yellow in all domestic animals. The yellow color is derived from bilirubin excreted into the intestine and reabsorbed into the portal circulation as urobilinogen [1].

## Urine collection

Methods of urine collection are the most important [2]. The urine can be collected by gentle tickling of the perineum around the vulva with a piece of straw or the fingers may encourage a cow or heifer to urinate. In male animals similar handling of the prepuce may be followed by urination. The Cattle very often will urinate during examinations, so have a suitable container ready. Continuous stroking of the skin just below the vulva of cows will usually induce urination. Once collected, the urine sample should be inspected, smelled and its contents tested [3]. Urine can be collected by catheter methods, in cow and heifers plastic catheter 0.5cm in diameter and 40 cm long may be passed into the bladder. The methods how catheter is inserted into bladder for collection of blood, first disinfection of vagina and placed gloved forefinger into suburethral diverticulum and insert catheter over the finger into urethra. Then urine may flow freely from the bladder into sterile syringe via catheter [3].

Horses both sexes urinate only while resting and cease feeding for the time and cows urinate similarly to mares, male cattle on the other hand urinate not only while feeding but also while walking, old dogs and pigs male void the urine in the interrupted jerky stream. Since bulls and steers cannot be catheterized, longer observation may be needed to obtain a sample. A collecting urinal may be strapped on male swine,

cattle, sheep, or goats to obtain a sample. Catheters can be used on both male and female horses. In male horses, manual pressure on the bladder via the rectum will sometimes induce urination. Close observation will enable collection from dogs and cats, but catheterization can be used successfully. Other collection methods from dogs and cats include applying pressure on the bladder and using a collection cage [4,5].

## Storage of Sampled Urine

Urine sample can be examined immediately after collection. However, the sample is expected to be delayed, the urine sample should be kept at 4°C without adding any preservative. For long preservative toluene can be added in urine to form a layer over urine, this is suitable for chemical examination. One drop of 40% formalin can be used as preservative in urine; however it may give false reaction for sugar examination [6].

## Physical examination of urine

The terms important in urination problem [1,7]:

- ✓ Polyuria refers to increase urine output
- ✓ Oliguria refers to decrease in urine output
- ✓ Anuria is a condition in which no output of urine.
- ✓ Dysuria is difficult or painful micturition.
- ✓ Stranguria is slow, dropwise, painful discharge of the urine caused by spasm of the urethra and bladder

Physical examination of urine includes assessment of color, clarity (transparency or turbidity), and specific gravity. Normal urine color, which varies from colorless or pale yellow to dark yellow, is associated with the presence of yellow pigments called urochromes, the end products of hemoglobin [7,8]. Cloudy urine usually is associated with presence of red blood cells, white blood cells, epithelial cells, crystals, casts, bacteria, lipid, mucus, or semen within the urine sample. Clear or slightly cloudy urine should not obviate microscopic examination of the urine sediment as abnormal findings are still possible [9].

Myoglobin is dark red colour heme containing protein, stored in muscle. Change in permeability of myolemma

causes myoglobin leak in plasma, which is cleared by kidney swiftly [10]. Myoglobin is released in the blood plasma as a result of damage to muscle tissue, because of its small size and lack of binding to haptoglobin, it is rapidly removed from blood by the kidney and excrete into urine. Hemoglobin, released when the red blood cell becomes hemolysis, rapidly and tightly bound to haptoglobin [11]. Generally, the urine can be examined by physical like examination of urine color, odour, consistence/turbidity, foaming and specific gravity [1,3].

### Examination of urine color and turbidity

The color of urine may be observed at the time of collection by keeping it in glass tube. The color of normal urine of domestic animals is water yellow to amber [12]. However, in certain disease conditions the color of urine alters as table below. Urine may be light to dark yellow and pale pink in color in bovines suffering from urolithiasis. Freshly voided urine from the healthy animals is usually clear, except in horse where it usually thick and cloudy due to the presence of calcium carbonates crystals and mucous [13].

Cloudy Urine is not Necessary Indication of Pathology in Horse [14]. Interestingly, in bovine obstructive urolithiasis urine may still be transparent and clear [15,16]. The variation in the colour of urine of the affected animals on day zero probably could be due to the variation in the concentration of urine, accumulation of sediments and haemorrhage. Dirty yellow coloured urine might be due to presence of sedulous materials in the urinary bladder. Brownish urine is indicative of mixing of blood in the urine, which could be due to haematuria or nephritis. Reddish colouration of urine is indicative of haematuria, which could be due to injury by calculi or inadvertent haemorrhage while performing surgery [17] (Table 1).

Normally the urine of equines is turbid and cloudy due to calcium carbonate and mucous while the urine of other animals is clear and transparent. Yellowish turbidity can be observed in pyogenic infections of kidneys. Kidney damage may be responsible for hematuria, albuminuria and proteinuria, which gives turbid urine. Cloudiness in urine is due to presence of leucocytes, erythrocytes, epithelial cells, bacteria, mucous, fat

and/or crystals. Of these, leucocytes give a white cloud while the red turbidity is seen due to erythrocytes and epithelial cells [2].

Bacteria form dorm cloudiness in urine and fat forms cloud. On addition of chloroform or ether in the urine sample urine becomes transparent. On centrifugation, the fat comes at the top of urine while other turbid material settles down [2].

### Odour of urine

The normal odor of urine is uremic, but some disease may change the odor of urine. Some of the example like, ketosis may produce sweetish or fruity odor of urine, pyogenic infection of kidney produce fetid odor. The abnormality sample may give the odour of ammonia, acetone or fetid odor [3]. Normal urine has a slight odor of ammonia; however, the odor depends on urine concentration. Some species, such as cats and goats, have pungent urine odor because of urine composition. Bacterial infection may result in a strong odor due to pyuria; a strong ammonia odor may occur if the bacteria produce urease [20].

### Specific gravity of urine

Specific gravity determined by number of particles per unity of solvent in time of ingestion of large amount of water specific gravity of urine as low as 1.001 is normal due to excess water for hemostasis. However, in the time of dehydration specific gravity of urine is increased due to marked conservation of water is expected. Specific gravity (SG) which is directly proportional to urine osmolality, measures solute concentration and urine density, or the ability of the kidney to concentrate or dilute the urine over that of plasma. Specific gravity in health varies with the state of hydration and fluid intake [15,17]. The range of specific gravity of urine in normal cattle is 1.025–1.045 with an average of 1.035 and in the obstructive urolithiasis it ranges from 1.008 to 1.025.

Under normal conditions, urine SG ranges between 1.015 and 1.040 in healthy dogs and between 1.036 and 1.060 in healthy cats [1,21]. An animal that is dehydrated or has other causes of prerenal azotemia will have hypersthenuric urine with an SG >1.025–1.040 (depending on species). Dilute urine in a dehydrated or azotemic animal is abnormal and could be caused by renal failure, hypo- or hyperadrenocorticism, hypercalcemia, diabetes mellitus, hyperthyroidism, and diuretic therapy. In cases of diabetes insipidus, values <1.010 can be expected (Table 2).

Glucosuria increases the SG despite increased urine volume [20]. The specific gravity increases in acute interstitial nephritis, cystitis, diabetes mellitus and dehydration and it decreases in chronic interstitial nephritis, pyometra and diabetes insipidus (Table 2).

### Foaming

On shaking, the normal urine produces white foams but in case of proteinuria, the amount of foam is in excess which remains for a longer duration. In icteric animals, the colour of foam may become green or yellow brown. The foams are red to brown in colour in case of hemoglobinuria.

**Table 1:** The abnormal color of urine with its cause ([www.veterinaryworld.org/Vol1.6/sept2013/11.pdf](http://www.veterinaryworld.org/Vol1.6/sept2013/11.pdf)).

Abnormality of urine	The color change of urine
Hematuria	Red colour urine, cloudy.
Hemoglobinuria	Brown to red colour.
Icterus	Yellow brown colour to greenish yellow
Pyogenic infections of Kidney	Yellowish green colour.
Azoturia in Horse	Brown to black colour of urine
Phenothiazine treatment	Red colour urine
Methylene blue treatment	Blue to greenish urine
Acridine treatment	Green coloured urine
Diabetes mellitus	Pale coloured urine
Diabetes insipidus	Pale coloured urine
Increased water up take	Pale coloured urine
Chronic interstitial Nephritis	Pale coloured urine
Pyometra	Pale coloured urine

## Chemical examination of urine

### Urine PH

The reaction of urine is determined by using pH strips or pH meter. Under normal conditions the urine of ruminants and horse is alkaline while in canines and feline it is acidic [22]. The pH of alkaline urine is 7.4–8.4, while that of acidic urine is 6–7. Urine becomes acidic during starvation, fever, treatment of ammonium chloride, sodium chloride, calcium chloride and sodium acid phosphate. The alkaline urine occurs in cystitis and due to treatment of acetate, bicarbonate; citrate and nitrates of sodium or potassium [22]. Urine pH will affect crystalluria because some crystals, such as struvite, form in alkaline urine, whereas other crystals, such as cystine, form in acidic urine [20,22].

Urine pH is a measurement of the kidneys ability to conserve hydrogen ions, thus it provides a rough but useful estimate of the body's acid–base status. However, urine pH does not necessarily reflect the body's pH, as it is highly influenced by diet, recent feeding, bacterial infection, storage time, metabolic and respiratory alkalosis, and urinary retention [23]. High protein diets, such as those consumed by carnivores produce neutral to acidic urine.

Herbivores tend to produce alkaline urine. Any animal may produce alkaline urine immediately after eating due to buffering that occurs in response to gastric acids. Alkaline nature of the urine is frequently linked to urinary tract infections. The bacteria break down urea and forms ammonia contributing towards the alkalinity of urine. Obstruction and renal tubular disease may also create alkaline urine. Acidic urine is commonly observed in animals with diabetes mellitus, especially if the animal is ketoacidotic. Excess or deficient dietary protein may lead to acidosis, as can Fanconi syndrome and metabolic acidosis [24]. The release of ammonia due to the breakdown of urea in the retained urine renders it alkaline. Struvite and calcium apatite uroliths are mostly found in urine with alkaline pH, while cystine stones are formed at the acidic pH. However, pH is variable in the formation of urate, silicate and calcium oxalate stones [25–27].

### Urine Glucose

The urine sugar or glucose is measured by the following method using Benedict's reagent. Take 0.5 ml of urine in a test tube and add 5.0 ml of Benedict's reagent and mix them thoroughly (Figure 1,4). Place this tube on a boiling water bath or flame for 5 min. Remove the tube from heating and keep on test tube stand for a few minutes and note the change of colour.

A urine dipstick test is a basic diagnostic tool used to determine pathological changes in patient's urine in standard urinalysis. A standard urine test strip may compromise up to 10 different chemical pads or reagent which react (change color) when immersed in and then remove from urine sample. The analyzing urine test is test for presence of proteins, glucose, ketones, haemoglobin, bilirubin, urobilirubinogen, and nitrite and leucocytes as well as testing Ph and specific gravity or test for infection by different pathogens (Figure 2,3).

Normally there should not be any glucose content in urine. Glycosuria occurs due to hyperglycemia and in diabetes mellitus, acute or chronic pancreatitis, hyperthyroidism, hyperadrenalism, hyperpituitarism, increased intracranial pressure, enterotoxemia. Certain drugs like streptomycin, chlortetracyclin, penicillin, tetracyclin and chloramphenicol may also lead to glycosuria [28]. False–negative results can occur with high urinary concentrations of ascorbic acid (vitamin C) or with formaldehyde (a metabolite of the urinary antiseptic, methenamine, which may be used for prevention of bacterial urinary tract infections). False–positive results may occur if the sample is contaminated with hydrogen peroxide, chlorine, or hypochlorite (bleach) [22].

Glucosuria in combination with hyperglycaemia reflects a tubular resorption defect in which the renal tubules fail to reabsorb glucose from the glomerular filtrate [29]. Nonpathologic glucosuria is associated with eating (postprandial), excitement

Table 2: The clinical identification of urinalysis [18].

Parameter	Clinical pathological condition
High specific gravity (>1.035)	Nephrotic syndrome, dehydration, acute glomerulonephritis, heart failure, liver failure.
Low specific gravity (<1.005)	Diabetes insipidus, nephrogenic diabetes insipidus, acute tubular necrosis.
Protein in urine	Renal disease, fever, congestive heart failure (CHF), hypertension, tumors.
Glucose in urine	Sugar, levels are obviously important in diagnosing diabetes.
Ketone bodies in urine	Ketonuria occurs in diabetes mellitus and starvation.
Bilirubin	Liver damage or disease.
Blood	Hemoglobin or myoglobin. Kidney damage, infection, kidney or bladder stones, kidney.
Cancer	Blood disorders, among other conditions.
Red blood cells (RBC's) in the urine	Haematuria
White blood cells (WBC's) in the urine	Infection, urinary tract infections (UTI)
Crystals	Hypercalcemia

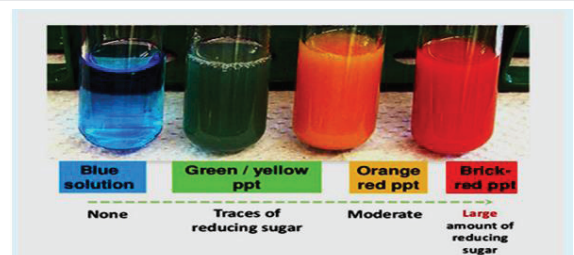
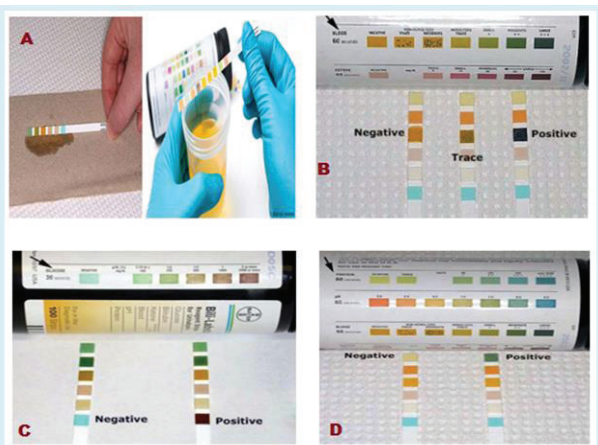
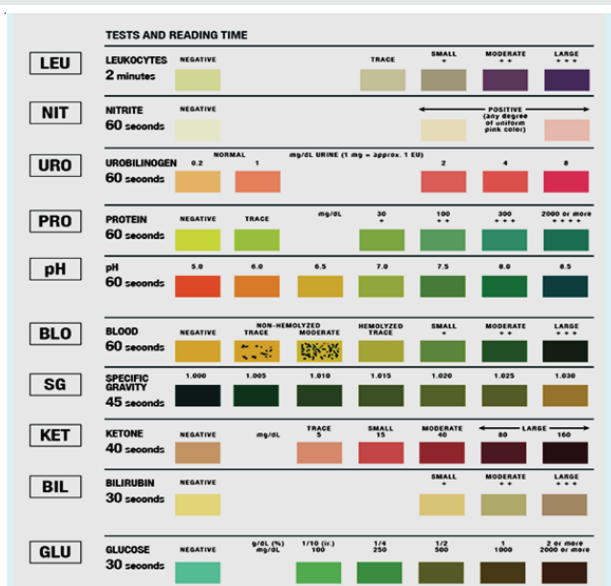


Figure 1: Benedictic test for glucose. Positive Benedict's Test: Formation of a reddish precipitate within three minutes. Reducing sugars present (Glucose). Negative Benedict's Test: No color change (Remains Blue). Reducing sugars absent (sucrose). <https://microbiologyinfo.com/benedicts-test-principle-composition-preparation-procedure-and-result-interpretation/>





**Figure 2:** Urine dip stick test. A : Removal of excess urine horizontally will prevent the mixing of chemicals from different reagent pads. B : Traces heraturia and marked hematuria or hemoglobinuria with Bayer reagent strips. C: Marked glucosuria with Bayer reagent strips. D : Marked proteinuria with Bayer reagent strips ([http://vetbook.org/cat/index.php?title=Urine\\_dip-stick\\_analysis](http://vetbook.org/cat/index.php?title=Urine_dip-stick_analysis)).



**Figure 3:** The reading and testing time of urine by dipstick testing method.

and stress (especially in cats and horses). Pathologic glucosuria is associated with diabetes mellitus, acute renal failure, and urinary obstruction in cats and milk fever in cattle. Numerous factors can decrease urine glucose values. These include refrigeration, ascorbic acid (vitamin C), salicylates, penicillin and presence of bacteria<sup>30</sup>.

### Protein

Urine protein levels are most conveniently determined with a dipstick. Normally, there is little to no protein present in urine. The presence of protein in urine is called proteinuria<sup>[21]</sup>. Proteinuria may result from glomerulonephropathy, tubular transport defects, inflammation or infection within the urinary tract. Increased protein level in the urine might be due to acute nephritis or inflammatory exudation resulting from pyelitis, urethritis, cystitis and urolithiasis <sup>[31]</sup>.

The glomerulus does not typically filter larger plasmaproteins, such as albumin and globulins, but it freely filters smaller proteins, which are reabsorbed in the proximal tubules of the kidneys unless there are significantly increased amounts of these proteins, or impairment of renal tubule reabsorption is present. The main protein in urine is albumin which comes under certain disease conditions. Robert's reagent test compositions are like; Nitric acid 1 part and saturated MgSO<sub>4</sub> 5 parts.

The procedure of Robert reagent test <sup>[32]</sup>:

Take 2 ml urine in the test tube, and then overlay it with 2 ml of Robert's reagent

Allow the urine to run slowly down along the wall of test tube. If albumin is present in the urine, a white ring will appear at the inter junction of two fluids which should be graded as follow: Very heavy ring (Highly positive), Heavy ring (Positive), A wide ring (Moderately positive), A narrow ring (Mild positive) and No ring (Negative).

Proteinuria may occur under some physiological conditions like excessive muscular exertion, stress, more protein in diet and convulsions which may disappear after removal of the factor. In disease conditions, proteinuria may occur in nephritis, acute interstitial nephritis, pyelonephritis and nephrosis. Phenol, arsenic, lead mercury, sulfonamides, phosphorus, turpentine and ether may cause proteinuria. Proteinuria may also occur in cystitis, prostatitis, pyelitis, urethritis, ureteritis, and urolithiasis and due to mixing of vaginal and prepuccial discharges in the urine <sup>[16]</sup>. The microscopic examination of urine is of great clinical importance. The important structures to identify include crystals, erythrocytes, leukocytes, casts and bacteria <sup>[33]</sup>.

### Ketone body test

Ketone bodies present in the urine react with the sodium nitroprusside which decomposed into sodium ferricyanide, ferric hydroxide and sodium nitrate. Then, these compounds in alkaline medium form complex with ketone bodies and produce purple colour. This colour is indication of positive test. Composition of Ross Reagent like Sodium nitroprusside 1 part and ammonium sulphate 100 parts <sup>[32]</sup>.

The procedure:

- Place a half inch layer of Ross reagent and add 5 ml of urine.
- Shake the 2 components
- Add 1-2 ml of ammonium hydroxide.
- Wait for five minutes. Development of purple color ring at junction is the indication of presence of ketone bodies.
- **Results:** Slight purple, Moderate purple, Dark purple and Dark purple to black color ring

### Bilirubinuria

The testing of bilirubin in urine is carried out by Gmelin test. The bile pigments are oxidized by acids and produced colored derivatives [32].

**Procedure:**

- Take 2 ml of nitric acid and 2 ml of urine in test tube.
- Presence of green to violet colour ring at the junction of two fluids is indication of presence of bilirubin in urine.
- **Result:** Bilirubinuria is seen in hepatocellular diseases like ICH (infectious canine hepatitis), leptospirosis, neoplasia; obstruction of bile duct; jaundice; and toxicides.

### Crystalluria examination in urine

Crystalluria is a frequent finding during the routine examination of urine sediments. In most instances the precipitation of crystals of calcium oxalate, monosodium urate crystals, triple phosphate, calcium phosphate and amorphous phosphates or urates is caused by transient super saturation of urine, ingestion of specific foods and also associated with pathological conditions such as urolithiasis, acute uric acid nephropathy, and ethylene glycol poisoning, and hyperesinophilic syndrome and due to some sulfa drug like sulphadiazine [34].

### Cellular evaluation of the sediments

Evaluation of the cellular components in the urine sediment is complicated by the fact that cells may originate from several areas such as the vascular system, interstitial tissue, urothelium or the genital tract (Table 3). The presence of increased number of white blood cells are evident in cystitis and pyelonephritis. Pyuria indicates a purulent process at some point in the urinary tract especially urethritis or cystitis [23].

### Test for haemoglobinuria, myoglobinuria and hematuria

The urine sediment is examined for the presence of white

**Table 4:** Benedict test for glucose in blood.

Color change of test	The degree of sugar
Blue (-)	No sugar
Blue to green sediment (+)	mild sugar
Yellow with heavy sediment (++)	moderate sugar
Orange with heavy sediment (++++)	Highly positive for sugar

blood cells, red blood cells, epithelial cells, crystals, bacteria, fat spermatozoa and fungi. High numbers of calcium carbonate crystals are expected in urine of equine. Squamous epithelial cells from the genital tract or distal urethra are often present in urine samples. Transitional epithelial cells originating from the more proximal urinary tract also observed.

Large clumps of transitional epithelial cells or cellular atypia should show the possibility of urinary tract neoplasia (transitional cell carcinoma). Blood reagent pad detects hematuria, hemoglobinuria and myoglobinuria. Intact erythrocyte produces a speckled color change, whereas hemoglobin and myoglobin produce a uniform color change [35]. Presence of partial hemolysis of erythrocyte in urine sample can indicate the combination of hemoglobinuria and myoglobinuria. If hemoglobinuria is due to hemoglobinemia rather than lysis of erythrocytes within urinary tract, clear red discoloration of the serum or plasma should be observed. Dark red urine not accompanied by hemoglobinemia or intact red cells on urine sediment examination is consistent with myoglobinuria. Myoglobin is a very small molecule with low renal threshold and discoloration of the plasma is not expected when there is muscle break down and release of myoglobin. There will be increase of muscle origin enzymes like creatine kinase and aspartate aminotransferase within myoglobinuria [35]. In sedimentation of urine if urine discoloration is due to hematuria, there will be numerous red blood cells and increased turbidity. However, if urine remains transparent when color change is due to hemoglobinuria [36,37]. Myoglobinuria and hemoglobinuria may cause a tea-colored appearance of the urine, and although both cause positive results on the urine dipstick for blood, myoglobinuria may be differentiated from hemoglobinuria by performing a series of simple tests. Myoglobinuria is brown, and often only a few RBCs are present in the urine. Hematuria produces a reddish sediment in spun urine samples. Red or brown urine with a negative dipstick result for blood indicates a dye in the urine. Hemoglobin produces a reddish or brown coloration in the spun serum, whereas myoglobin does not discolor the serum. CK levels are markedly elevated in myoglobinuria. Results of radioimmunoassay for the specific measurement of serum or urine myoglobin can be delayed by several days and are not useful in immediate diagnosis and treatment [38].

The myoglobinuria may be clinically detectable as a red-brown or chocolate-brown discoloration of the urine. This discoloration can be differentiated from that caused by hemoglobin by spectrographic examination or with the use of orthotoluidine paper strips. Urine becomes dark when myoglobin levels exceed 40 mg/dL of urine [39]. Hemolysis indicates some intravascular hemolysis and myoglobinuria is indicated by muscle pathology [40].

**Table 3:** Types and characteristic of urinary cast [19].

Casts in urine	Source of cast	Causes of presence of cast in urine
Protein	Mucoprotein	Normal health, fever, exercise, diuretics, renal disease
Granular	Degenerating cellular casts	Glomerular disease, tubular disease, pyelonephritis, viral infections or aggregated proteins
Waxy	Final stages of granular cast	Advanced renal failure or other conditions with dilated tubules with diminished flow degeneration
Fatty	Lipid-containing renal	Nephrotic syndrome tubular cells
RBC's	Red blood cell	Glomerulonephritis, Tubulointerstitial nephritis, acute tubular injury/necrosis
WBC	White blood cells	Pyelonephritis, glomerulonephritis, Tubulointerstitial nephritis
Epithelial cell	Renal tubular cells	Epithelial cell Renal tubular cell

The pattern of increase in muscle enzymes and myoglobin concentrations in horses with RER suggested that the high plasma AST and CK activities commonly observed at rest in symptom-free Standard bred horses are probably a result of repeated subclinical episodes of rhabdomyolysis after exercise, rather than leakage due to abnormal sarcolemmal permeability [41]. Myoglobinuria is a common finding in adult horses with acute paralytic myoglobinuria but is not a common finding in acute nutritional muscular dystrophy in young farm animals, except perhaps in yearling cattle with acute muscular dystrophy.

White muscle disease is associated to vitamin/selenium deficiency but it is exacerbated by other factors such as exercise, environment (climatologic conditions may be involved), nutrition and some toxicants and also myoglobinuria may cause by necrosis of skeletal muscle in horse like azoturia (paralytic myoglobinuria, Monday morning disease, sacral paralysis). It is a disease of horses associated to strenuous exercise after a long resting period and a diet rich in carbohydrates [42,43].

### Hematuria

Hematuria is the presence of intact blood in the urine which appears as gross blood clots passed at the beginning (caused by urethral damage), after exercise in horse (caused by cystic calculi), or at the end of urination (caused by vesicle damage) or as more uniformed discoloration of the urine throughout the urination without clots. If the blood is equally distributed in the urine, it could be caused by kidney damage. At the centrifugation or sedimentation of urine the RBC is sedimented and the upper part become clear no red colouration [40,45] (Figure 4). The most causative agent of hematuria are classified as prerenal causes (trauma to kidney, septicemia and purpura hemorrhagica in horse), Renal causes (acute glomerulonephritis, pyelonephritis, tubular damage due to sulphonamide toxicity, embolism and renal infarction), Post renal causes (urolithiasis, urethritis, cystitis and enzootic hematuria in cattle, tumor in urinary bladder [35,44].

### Hemoglobinuria

Hemoglobinuria is the presence of hemoglobin in the urine which caused by hemolysis of RBC. The true haemoglobinuria is manifested by deep red discoloration of the urine caused by lysis effect of *Bacillary hemoglobinuria*, *babesiosis*, copper intoxication, water intoxication on RBC [46]. False hemoglobinuria is occurs with cases of hematuria, when RBC are destroyed and liberate their contents of hemoglobin into urine.

Generally, to differentiate hemoglobinuria from myoglobinuria and hematuria (Figure 5,6), which all have a positive blood test on a urine dipstick, evaluate the color of the supernatant after centrifugation of the urine; hematuria will have a clear supernatant, whereas hemoglobinuria and myoglobinuria will not. To differentiate hemoglobinuria from myoglobinuria, evaluate the plasma color; hemoglobinuria will have a pink to red plasma color, whereas myoglobinuria will not [47]. If necessary, ammonium sulfate precipitation or urine protein electrophoresis can be used to differentiate



Figure 4: Centrifugation result of hematuria.

([http://www.youpetsbestfriend.com/your\\_pets\\_bes\\_friend/2008/07/bladder-blockage.html](http://www.youpetsbestfriend.com/your_pets_bes_friend/2008/07/bladder-blockage.html)).



Rapid test to distinguish hematuria from hemoglobinuria. The onset of red urine during or shortly after a blood transfusion may represent hemoglobinuria (indicating an acute hemolytic reaction) or hematuria (indicating bleeding in the lower urinary tract). If freshly collected urine from a patient with hematuria is centrifuged, red blood cells settle at the bottom of the tube, leaving a clear yellow urine supernatant. If the red color is due to hemoglobinuria, the urine sample remains clear red after centrifugation.

Figure 5: The urine test which distinguish hematuria form hemoglobinuria.

(<https://emedicine.medscape.com-artical/206885-ocerview>).



Figure 6: The differentitation of hemoglobinuria from hematuris.

Red-brown discoloration of the urine suggests hemoglobinuria, hematuria, or myoglobinuria ; however, red discoloration of the plasma (right) indicates that hemoglobinuria is persent and this produced the hemeoglobinuria (<http://vetbook.org/wiki/cat/index.php?title=/Hemoglobinuria>).

hemoglobinuria from myoglobinuria [42]. Ammonium sulfate precipitation test, 5 ml of urine is mixed well with 2.8 mg of ammonium sulfate and centrifuged. Hemoglobin precipitates, myoglobin does not and if the supernatant remains dark after centrifugation, suspect myoglobinuria [48].

### Myoglobinuria

Myoglobinuria is the presence of myoglobin in the urine. The myoglobinuria may be clinically detectable as a red-brown or chocolate-brown discoloration of the urine. Myoglobinuria



is caused by massive muscle necrosis (rhabdomyolysis) [49]. Myoglobinuria caused by acute myositis (e.g., toxoplasmosis), Compartment syndrome, Crush injury, Extreme exercise (rhabdomyolysis), and Tornequet syndrome prolonged Seizures. Myoglobinuria is usually the result of rhabdomyolysis or muscle destruction. Any process that interferes with the storage or use of energy by muscle cells can lead to myoglobinuria.

The release of myoglobin from muscle cells is often associated with an increase in levels of creatine kinase (CK), aldolase, lactate dehydrogenase (LDH), serum glutamic-pyruvic transaminase (SGPT), and other enzymes. When excreted into the urine, myoglobin, a monomer containing a heme molecule similar to hemoglobin, can precipitate, causing tubular obstruction and acute kidney injury

Myoglobin is a very small molecule with low renal threshold and discoloration of the plasma is not expected when there is muscle break down and release of myoglobin. There will be increase of muscle origin enzymes like creatine kinase and aspartate aminotransferase with in myoglobinuria. This enzyme can measure the urine discoloration is due to myoglobin [35]. Hemolysis indicates some intravascular hemolysis and myoglobinuria is indicated by muscle pathology [40]. The pattern of increase in muscle enzymes and myoglobin concentrations in the horses with RER suggested that the high plasma AST and CK activities commonly observed at rest in symptom-free Standardbred horses are probably a result of repeated subclinical episodes of rhabdomyolysis after exercise, rather than leakage due to abnormal sarcolemmal permeability [41,42].

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The myoglobinuria may be clinically detectable as a red-brown or chocolate-brown discoloration of the urine. This discoloration can be differentiated from that caused by hemoglobin by spectrographic examination or with the use of orthotoluidine paper strips. Urine becomes dark when myoglobin levels exceed 40 mg/dL of urine [42]. As the cell breaks down, large quantities of Potassium, aldolase, phosphate, myoglobin, CK, lactate dehydrogenase (LDH), aspartate transferase (AST) and urate leak into the circulation [50]. When more than 100g of muscle tissue is degraded the plasma's myoglobin binding capacity is overwhelmed and free myoglobin causes renal morbidity by several mechanisms [51].

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