

Covid Research and Treatment

Research Article

Importance of Urinalysis In Vetirinary Practices: A Review

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Received Date: 20 March 2026; **Accepted Date:** 21 April 2026; **Published Date:** 25 May 2026

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Abstract

Urinalysis was the initial laboratory examination conducted in medicine and has been utilized for thousands of years. Currently, it remains an effective resource for acquiring essential information for diagnostic objectives in healthcare. Urine is a volatile liquid/ fluid and alterations in its makeup start immediately after it is expelled. Consequently, gathering, preserving, and managing are crucial aspects in maintaining the integrity of this specimen. Primarily, there are three techniques used for gathering urine samples: Cystocentesis, Catheterization, and mid-stream free flow. A urine sample can be assessed for its physical characteristics, chemical composition, enzyme levels, and sediment (microscopic) analysis. Urinalysis is an impressive method that can uncover numerous diseases that might remain unnoticed and undiagnosed, as they typically do not exhibit prominent signs or symptoms. Examining the color, clarity, microscopic and chemical properties of urine and urinary sediments along with microbial culture and sensitivity testing is an effective method for diagnosing lower urinary tract infections and metabolic disorders in domestic animals. Urinalysis is an underused aspect of veterinary medicine today, even though it is a readily available and cost-effective method for identifying and managing various urinary tract issues and metabolic conditions. The aim of this seminar is to examine different types of literature that help veterinarians grasp the significance of urinalysis in diagnosing specific diseases and to motivate veterinarians to incorporate this useful and straightforward tool into their practice for certain disease diagnosis.

Keywords: Urine, Urinalysis, Veterinary Medicine.

Introduction

The renal system includes the organs involved in producing and eliminating urine: these are the kidneys, ureters, urethra, and urinary bladder [1] These organs are crucial for removing metabolic by-products and waste materials, maintaining water balance, controlling red blood cell production, and managing blood pressure.[2] Urinary tract infection (UTI) occurs more frequently in companion animals compared to other domestic species and contributes significantly to the antimicrobial resistance observed in both human and veterinary medicine.[3] A better comprehension of UTI pathophysiology, the risk factors for clinical disease, and the application of more reliable diagnostic tests can enhance treatment results and decrease improper treatments that contribute to

drug resistance.[4]

Urine has emerged as one of the easiest bio fluids to obtain since it can be gathered non-invasively in significant amounts.[5] It is formed through the integral process of ultrafiltration of glomerular filtration, the return flow of essential molecules-tubular absorption, and the incorporation of additional waste substances into the renal tubules - tubular secretion.[6] Urinalysis is the initial laboratory test conducted in medicine and has been utilized for thousands of years. Currently, it remains an influential instrument for acquiring essential data for diagnostic aims in healthcare. It is among the essential instruments available to Veterinarians that significantly contribute to diagnosing certain diseases globally,

including in Africa and Ethiopia.[7] However, in many countries including Ethiopia, it remains a largely neglected aspect of veterinary medicine.[8] Urinalysis aids in assessing both normal and abnormal components of urine. It is a simple, inexpensive, and crucial preliminary diagnostic test for veterinarians. A Complete urinalysis involves evaluating physical traits, biochemical factors, microscopic sediment, and enzyme assessment (it entails assessing color, smell, clarity, volume, pH, specific gravity, protein, glucose, ketones, blood, erythrocytes, leukocytes, epithelial cells, casts, crystals, and organisms).[4]

Urinalysis ranks as the third primary diagnostic screening test in clinical laboratories, following serum/plasma chemical profiles and complete blood count analysis. It is a crucial assessment for the presence, severity, and duration of urinary tract disorders.[9-4] It is the most effective method for identifying kidney problems prior to renal failure, and a thorough urinalysis can assist in revealing several metabolic conditions like ketosis, diabetes, liver issues, and intravascular hemolysis by measuring glucose, ketones, bilirubin, and elevated hemoglobin levels.[4] Among the various techniques used for urine collection in veterinary settings, free catch, which involves obtaining mid-stream urine during natural urination, is the most prevalent method, while manual bladder compression, catheterization, and cystocentesis are also employed for collecting urine samples. The urine sample must be assessed within 30 minutes. However, if this cannot be done, it should be stored in the refrigerator for a maximum of 24 hours.[10-4]

Urinalysis offers valuable insights regarding the presence or absence of renal and other disease conditions, and as a standard test, it serves as a straightforward technique for tracking disease progression and the effectiveness of therapy.[11]

Unfortunately, it receives minimal attention or is a largely neglected element in the current veterinary medicine; hence this effort aims to examine the different facets and benefits of urinalysis in veterinary practice and to motivate veterinarians to employ this essential tool in diagnosing specific diseases more easily, which would otherwise require complicated procedures.

Hence, the goals of this seminar are: to examine different types of literature for veterinarians to grasp the significance of urinalysis in diagnosing specific diseases. Additionally, to motivate veterinarians to employ this essential and straightforward tool in their practice for diagnosing certain diseases.

Review on the Importance of Urinalysis In Veterinary Practices

Urine Sample Collection

Urine tests indicate the condition of the urinary system, primarily the kidneys, as well as other associated systems.

[7] Therefore, adequate sampling and storage (if required) are essential for any precise prediction of a disease. Mainly, three techniques are used for collecting urine samples: Cystocentesis, Catheterization, and mid-stream free flow.[7]

Cystocentesis

Among the three available methods, cystocentesis is the most favored and precise option. It is the preferred technique for urine bacterial culture and can be conducted without requiring sedation for patients.[12] A sterile needle is introduced through the abdominal wall into the urinary bladder to extract urine from the storage (bladder). The primary benefit of this method is obtaining a contamination-free sample, which is beneficial for identifying bacterial infections in the urinary bladder and kidneys.[13]

Catheterization

Another method utilized in practice for urine sample collection involves a sterile and meticulous technique to maintain the integrity and health of the patient's urinary tract, allowing urinary bacterial cultures to be conducted without the proliferation of contaminant bacteria.[14] Catheterization enables the collection of urine without requiring a distended bladder and is independent of the animal's readiness to urinate.[15] Typically, a narrow sterile catheter is inserted into the urethra until it reaches the bladder, causing urine to begin dripping from the catheter's end. This method could lead to new bacterial infections entering the urinary bladder through the urethra.[4]

Mid-stream free flow

Natural micturition is the most commonly employed technique in bovines, allowing for easy non-invasive collection. It can serve as an initial screening test or be conducted by owners to evaluate the progression of a disease through urinalysis.[14] The animal voluntarily expels urine in the normal manner, and a sample is gathered in a sterile container during urination. Ideally, the sample should be taken during mid-stream, which is partway through urination. This kind of sample is frequently referred to as a "free flow" or "free catch" sample. This method's benefits include being entirely non-invasive, and the pet owner can gather the urine sample at home. The drawbacks include a heightened risk of urine contamination from debris in the urethra or surrounding environment.[16].

Preservation of Urine Sample

Ideally, it is suggested that examination occurs within 30 minutes after collecting a fresh sample. However, if that's not feasible, urine must be preserved using either physical or chemical methods. Physical method: Primarily achieved

by refrigeration immediately and should not be kept for over 6-12 hours. Before examination, refrigerated samples must be well mixed to re-dissolve any substances that may have crystallized at cold temperatures.[17].

Chemical Method: The incorporation of ethylene diamine tetra acetic acid (EDTA), Hydrochloric acid (HCl), Chloroform, Boric acid, Sodium carbonate, and formalin can serve as preservatives for urine.[18]

Examination of Urine

A thorough urine analysis entails both a macroscopic and microscopic evaluation of the urine specimen. A macroscopic analysis is relatively fast, inexpensive, and straightforward. [18-19].

It comprises a subjective evaluation of the physical characteristics (color, smell, clarity, and volume) along with a specific gravity measurement. It also encompasses a semi-quantitative evaluation of urinary chemical properties through dipstick analysis.[4]

Microscopic assessment primarily concentrates on analyzing urine sediment and involves identifying crystals, cells, casts, and microorganisms to indicate potential pathological conditions. [20-21].

Physical examination of urine

Color and transparency: The color of urine is assessed by observing the urine in a test tube against a white background under adequate lighting or within a urinometer cylinder. Urochrome is a combination of urobilin and urobilinogen with peptide, serving as the pigment responsible for the normal color of urine [22-04].

The Normal color of urine varies from light yellow to amber and is influenced by the level of Urochrome present.[21], Nonetheless, a dark yellow color in dogs and cats is frequently linked to dehydration from vomiting or diarrhea, while a very pale yellow color might indicate polydipsia or polyuria, suggesting potential underlying kidney issues or a failure to concentrate urine.

Urine that is brown in color indicates possible haemoglobinuria or nephritis. Faint pink color of urine suggests congenital porphyria or urolithiasis.[21-23].Typically, the urine of all animals is transparent, clear, and uniform, except for that of horses [24].

The presence of epithelial cells, calcium carbonate crystals, and amorphous urate may obstruct this condition. In horses, it is common to see cloudy urine because of a minor rise in mucous and calcium carbonate crystals present in the urine [25].

Volume of urine: The amount of urine produced is influenced by hydration levels and the kidneys' concentrating ability. It is negatively correlated with the specific gravity.[4].

Animals	Volume of urine
Cats	18-25 ml/kg/day
Kittens	5-60 ml/kg/ day
Dogs	14-50 ml/kg/day
Sheep and goat	10-40 ml/kg/day
Human	1-2L/day
Horse	8-30 ml/kg/ day

Source (Reece et al., 2015).

Table 1: Normal urine volume on different animals

Specific gravity: The specific gravity (USG) reflects the weight ratio of 1 L of solution to 1 L of water and offers important insights into the kidney's capacity to concentrate or dilute urine [26].

Specific gravity is inversely related to urine volume, but exceptions occur in individuals with diabetes mellitus, who excrete large amounts of urine with elevated specific gravity. Urine with a specific gravity under 1.007 is classified as hyposthenuria, while a specific gravity greater than 1.012 (USG >1.012) shows that the kidneys have some ability to concentrate the glomerular filtrate. A specific gravity between 1.008 and 1.012 indicates hypersthenuria and isosthenuria for plasma and glomerular filtrate-specific gravity, respectively [4].

USG in healthy cats and dogs falls between 1.001-1.085 and 1.001-1.075, respectively [27].However, the specific gravity of urine can be inaccurately elevated by 0.003 to 0.005 for each 1g/dL of protein present in the urine and by 0.004 to 0.005 for every 1g/dL of glucose in urine [21].The specific gravity of urine can be measured using a urinometer or a refractometer. A urinometer is a type of hydrometer with scale markings that align with the specific gravity range found in urine. It is essentially consists of a weighted float with a slender graduated stem that immerses in the test liquid to a depth corresponding to the liquid's specific gravity.

A refractometer measures the concentration of a specific substance in a solution. It operates according to the principle of refraction. When light rays transition from one medium to another, they are refracted either toward or away from the normal line that separates the two media. Various refractometer devices are commercially available, offering four measurement scales: Canine USG, Feline USG, Large Animal USG, and Serum Protein concentrations applicable to all animals (Figure 1)[28]



Refractometer

Source: Anonymous (2022) Urine Specific Gravity Test: Procedure, (Normal Range, High, Low) Clinical Significance.

Figure 1. Device used for measuring of urine specific gravity.

Odor: The typical smell of urine is characterized as sui generis (offensive odor), resulting from the volatile fatty acids found in urine [29]. Urine components are both intermediate and final products of various metabolic pathways, featuring numerous structural patterns like ketones, alcohol, and sulfide, which frequently produce a distinct odor. In specific cases, characteristic urine smells were directly associated with certain biochemical conditions, and the origins of these odors were identified.[30]

An ammonium smell is noticeable when urease-producing bacteria are present, while a fruity aroma and sweet smell can be sensed due to the existence of acetone or ketone bodies and glucose or other sugars, respectively. A foul smell may be detected in samples that have been kept for an extended duration as a result of protein decay [29]. Nonetheless, the odor of urine may not be regarded as indicative of any particular disease [18-13].

Chemical examination of urine

PH of Urine: Urine pH provides a general indication of how well the kidneys can concentrate hydrogen ions and serves as a tool for veterinarians to assess the overall acid-base balance of the body. Herbivores typically generate alkaline urine, whereas carnivores produce acidic urine [20-31]. Nutrition can influence acid-base balance through the intake of acids and bases from food. Hepatic oxidation of sulfur-containing amino acids like methionine and cysteine produces

hydrogen ions[31]. This is counterbalanced by carbonates from green plants/vegetables that provide greater quantities of Magnesium and Potassium to sustain balance. The primary organs for excreting dietary hydrogen ions are the kidneys. Therefore, it is directly observable in the urine. Dogs with urinary tract infections frequently exhibit alkaline urine. [32].

Numerous techniques exist for measuring the pH of urine: litmus paper, pH indicator paper, pH meters, and reagent strip tests. Litmus paper examination: A tiny piece of litmus paper is immersed in urine, and any alteration in color is recorded. When blue litmus paper changes to red, it signifies acidic urine. If the red paper changes to blue, it signifies alkaline urine.

pH indicator paper: The reagent section (infused with bromothymol blue and methyl red) of the indicator strip is submerged in the urine sample, and the color shift is matched against the color chart supplied by the manufacturer. **pH meter:** An instrument equipped with a pH measuring electrode is inserted into the urine sample, and the pH value is displayed directly on the digital screen.

Reagent strip test: The test region has polyionic polymer attached to H^+ ; when it reacts with urine cations, H^+ is liberated, resulting in a color shift of the pH-sensitive dye (Figure-2) [33]



(A) Litmus paper, (B) reagent strip test, (C) pH indicator paper and (D). PH meter

Source: <https://labtests.co.in/urine-ph-test/> Urine pH test: Procedure, Causes (Normal, Low, High) and Clinical Significance Accessed september 05, 2024.

Figure 2: Device used for testing pH of urine

Proteins in urine: A healthy animal will almost eliminate no protein, but will definitely excrete less than the threshold for regular urine protein detection [34]. An excessive level of proteins in urine is referred to as Proteinuria. Glomeruli stop large protein particles like albumin and globulin from being excreted in urine, but under physical oxidative stress, especially in the renal system, this may result in albuminuria. In addition to this, several other forms of stress, whether physical or emotional, can cause heightened glomerular permeability, ultimately leading to Proteinuria [34-35]

The presence of protein in urine can be identified using a dipstick that mainly evaluates albumin levels [36]. Dipstick identifies protein through color change using an indicator dye. Bromophenol blue indicator is a widely used test for protein since it is highly sensitive to albumin and can also detect globulin, like Bence-Jones, albeit poorly. The Sulfosalicylic acid test is an effective method for detecting albumin and globulin, even in low concentrations.

The most precise technique for diagnosing proteinuria involves calculating the protein: creatinine ratio. Another

technique is Robert's method, wherein strong acids interact with proteins, causing them to precipitate out of the solution; the presence of protein is signaled by the appearance of a white ring or precipitate at the interface of the reagent and urine.[37]

Glucose in urine: A healthy animal excretes minimal to no glucose in its urine. Glucose can be present in urine when blood sugar exceeds the renal threshold or if renal tubular reabsorption is impaired [38]. It is filtered freely and subsequently reabsorbed in the proximal tubules, thereby conserving glucose for energy use. The occurrence of glucose in urine is termed Glucosuria. When blood glucose levels are excessively high (hyperglycemia), they surpass the renal threshold for reabsorption, resulting in glucose being eliminated in urine. The renal threshold for dogs is 10mmol/L, whereas in cats, it ranges from 14 to 17mmol/L [35-39]. Non-pathologic glucosuria occurs after eating (postprandial), as well as during excitement and stress (particularly in cats and horses). Pathologic glucosuria is related with diabetes mellitus, acute renal failure (renal dysfunction), hyperthyroid-

ism, hyperactivity of adrenal cortex and blockage.[35] Different tests are utilized for the identification of glucosuria. Among these, the non-specific reduction test (Benedict test) and enzymatic tests utilizing glucose oxidase (strip or colorimetric methods) are the most prevalent techniques in urinalysis for diagnosing diabetes mellitus, hyperthyroidism, hyperadrenalism, hyperpituitarism, and certain pancreatic diseases [40] Chemical strips identify glucose via an enzyme chemical reaction that causes a color change related to the glucose concentration present. The outdated strip could provide a misleading negative result. Urine temperature may influence the outcome, thus refrigerated samples must be returned to room temperature.[4] Glucosuria usually occurs when the blood glucose concentration exceeds around 200 mg/dL in dogs and 250-300 mg/dL in cats.[40]

Ketone in urine: Ketones result from fat metabolism, with a normal reference range of zero to negative. The presence of ketone bodies in urine is known as ketonuria (Jacob, 2020). [21] Ketonuria is a frequent pathological condition; particularly in ruminants, it can signify diabetic ketoacidosis. Animals in the late stages of pregnancy and early post-delivery may experience ketonuria. Diets rich in fats, excessive starvation may also lead to ketonuria.[21]. Ketones are usually produced in minimal quantities that cannot be detected in urine. [41] Commercially available options for ketone monitoring include nitroprusside reagent test strips (chemical strips) and a Beta-hydroxybutyrate ketone meter. Ketones are evaluated in the chemical strip via the legal reaction. This reaction depends on the interaction of acetoacetate with nitroprusside in an alkaline medium as observed in urine.[4-42]

A positive reaction is identified through a color change, varying from lavender to deep purple, using a reagent strip or a chemical method with nitroprusside. All nitroprusside reagent strips can detect acetone and acetoacetate. Nonetheless, they are significantly more responsive to acetoacetate compared to acetone.[42]. Ketonuria indicates a shift from carbohydrate metabolism to fat metabolism. This change is primarily recognized in small animals experiencing ketosis due to diabetes mellitus, though hunger also results in elevated ketone levels. These factors are affected by metabolic requirements that exceed what carbohydrate metabolism can supply.[13]

Bilirubin in urine: Upon the breakdown of hemoglobin, the heme component is transformed into bilirubin, which is processed in the liver and eliminated in bile.[43] A portion of conjugated bilirubin is filtered through the glomerulus and eliminated in urine. The standard reference range for bilirubin is zero to negative. The bilirubin threshold range is minimal, so even a slight variation in plasma bilirubin can result in bilirubinuria. Bilirubinuria is sometimes observed in dogs, attributed to a low renal threshold and because canine renal tubular cells can break down hemoglobin into unconjugated

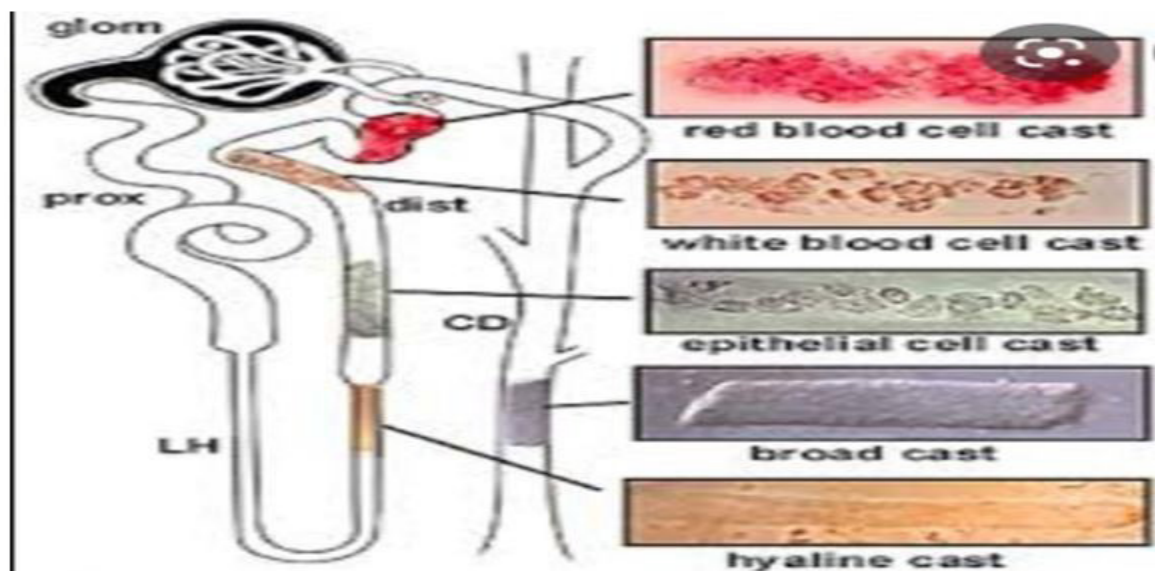
bilirubin and subsequently excrete it into urine.[21] In contrast to dogs, bilirubinuria in cats is linked to various underlying conditions. It is linked to feline infectious peritonitis, primary liver disorders, diabetes mellitus, and conditions related to feline leukemia. Bilirubin is sensitive and reduced by sunlight exposure and elevated vitamin C levels.[21] Bilirubin tests rely on the oxidation of bilirubin to biliverdin, requiring specimens that are freshly voided to avoid the degradation of analytes caused by sunlight exposure. Harrison's (Fouchet's) test and the Gmelin test are the most commonly employed methods that use acids to oxidize bile pigments. They are essential tools for identifying complete and partial biliary obstruction, hepatitis, hemolysis, acute enteritis, and intestinal obstruction that relate to the biliary system.[44]

Microscopic examination of urine

Microscopic analysis of urine is a crucial aspect of urinalysis and holds significant clinical importance. Urine microscopy includes counting and recognizing insoluble components in urine. It can be conducted on wet mounts, air-dried smears, or both.[18] When placed in a centrifuge, urine sediments fall to the bottom of the tube. Typically, casts and erythrocytes vanish or break down when urine is stored for an extended period. Therefore, it is advised to use newly obtained samples for sediment analysis.[21]

Casts: Casts are long, cylindrical structures formed by mucoprotein solidifying inside renal tubules and may include cells. Various types of casts are produced in different regions of the renal tubules: hyaline cast, granular cast, epithelial cast, waxy cast, fatty cast, blood cast, and leukocytic cast. [41] Hyaline casts are clear protein deposits that feature straight sides and rounded ends, composed of mucoprotein. They can arise with fever, physical activity, and kidney disease. Epithelial cellular casts result from the entrapment of shed tubular epithelial cells within mucoprotein and can be observed in renal tubular disease.[45]

Granular casts are believed to indicate deteriorated epithelial cell casts. Waxy casts display a granular look and are believed to result from the degeneration of chronic granular casts. They usually possess defined edges with fractured tips.[46] Additional cellular casts consist of RBC casts and WBC casts, which are consistently abnormal. RBC casts are formed due to kidney bleeding. WBC casts develop due to kidney inflammation, as seen in pyelonephritis. Fatty casts are rare but may appear in conditions affecting lipid metabolism, like diabetes mellitus. A small number of hyaline or granular casts is regarded as normal.[47]. The occurrence of cellular casts or other casts in significant quantities suggests kidney injury and may represent one of the initial lab abnormalities observed with toxic harm to renal epithelial cells (e.g., gentamicin, amphotericin B).[45]



Source: Pathology of Case Studies: www.medscl.indiana.edu

Figure 3: Different types of cast and their site formation in the renal tubules

Crystalluria: The presence of crystals in urine is a common observation and is not always linked to disease. [48] They are commonly present in urine, with their formation relying on the oversaturation of the mineral substrate, urine pH, and the duration between urine collection and analysis. Urinary crystals in herbivores hold no particular importance unless they are present in significant quantities and are accompanied by additional clinical symptoms like urinary tract irritation. Crystalluria of calcium oxalate and calcium carbonate is frequently observed in healthy cattle and horses. Small amounts of bilirubin crystals can be normal in dogs. Certain types of drugs like sulphadiazine, trisilicate, ciprofloxacin, and indinavir can

also lead to the formation of crystals.[21]

Various crystals possess distinct shapes. Struvite crystals resemble coffin lids; calcium oxalate monohydrate crystals appear like picket fences, featuring envelope ditetragonal pyramids or bipyramidal forms.[21] Calcium oxalate dihydrate crystals exhibit a Maltese cross or square envelope form. Urate crystals are shaped like thorn apples or fine needles, while cystine crystals have a hexagonal structure. Struvite and calcium phosphate crystals can be seen in the urine of dogs and cats when the pH is neutral to alkaline. Urine with a low pH can contain amorphous urate, cysteine urate, calcium oxalate, and hippuric acid. [49]



Source: <https://pubmed.ncbi.nlm.nih.gov>

Figure 4: Different Crystalluria formed in the renal tubules

Conclusion and Recommendations

Urinalysis is a reliable screening procedure that can provide extensive information about the urinary system and associated metabolic processes, assist in the empirical diagnosis of diseases, track the progression of illnesses, and evaluate treatment effectiveness when conducted correctly. It is a secure, non-invasive technique, an easy analysis of urine that necessitates only urination from the participant; it causes no discomfort, presents no health-related risks, has no immediate side effects, and shows no negative reactions. Nevertheless, the techniques used for sample collection and management are essential for result interpretation, so utmost caution is necessary. Ultimately, all the physical, chemical, and microscopic observations must align to interpret the outcome. Additionally, it is important to connect these results with the medical history and physical examination of the case to gain a comprehensive understanding. Urinalysis is vital for the prompt detection of various medical conditions and can facilitate early treatment and improved recovery rates for specific urinary and metabolic disorders. We determine that most urinalysis techniques are reliable indicators of urine-culture diagnosis and can serve as effective diagnostic instruments for Urinary Tract Infection. However, even if it is easily accessible and a cost-effective tool for diagnosing and managing specific diseases, it remains a largely neglected aspect in veterinary medicine.

Therefore, based on the above conclusion, the following recommendations are forwarded:

- All veterinary professionals should be motivated to utilize urinalysis techniques in various laboratory set up/ environment. All relevant concerned bodies should provide all necessary materials and reagents to implement these techniques at the appropriate standard.
- It is essential to raise awareness among stakeholders regarding the significance of urine analysis.

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