GOING FOR THE GOLD: URINALYSIS AND BEYOND
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• Part of a minimum data base
• Evaluate with urinary disease: “God made it gold for a reason”
• Collect by void, cystocentesis, catheterization
  • Evaluate within 30 minutes
  • If not possible, refrigerate (2-8°C) up to 24 hours
  • Re-warm to room temperature
• Delay may alter results
  • Bacterial contamination or death
  • Glucose utilization
  • False increase in pH and protein
  • Casts and cells deteriorate
  • *In vivo* precipitation of crystals
• Components of complete urinalysis
  • Urine appearance
    • Color
      • Normal urine is transparent and yellow or amber
      • Intensity of color is related to volume of urine collected and concentration of urine
      • Interpret in context of urine specific gravity (USG)
      • Disease may exist with normal color
      • Abnormal color may be caused by presence of endogenous or exogenous pigments, but it does not provide specific information. Interpretation of semi-quantitative reagent strips, which are colorimetric tests, requires knowledge of urine color because discolored urine may result in a false positive result. Equine urine may turn brown after a period of time.
    • Red urine does not mean blood (hematuria)
      • Positive OCCULT BLOOD on test strip – can be blood, hemoglobin, or myoglobin
      • Pigment: e.g. myoglobin (clear serum) or hemoglobin (red serum)
      • Other pigments: e.g. drugs
## Table. Potential causes of discolored urine

<table>
<thead>
<tr>
<th>URINE COLOR</th>
<th>CAUSES</th>
<th>URINE COLOR</th>
<th>CAUSES</th>
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</thead>
<tbody>
<tr>
<td>Yellow or amber</td>
<td>Urochromes</td>
<td>Yellow-brown or green-brown</td>
<td>Bile pigments</td>
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<td></td>
<td>Urobilin</td>
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<tr>
<td>Deep yellow</td>
<td>Highly concentrated urine</td>
<td>Brown to black (brown or red-brown when viewed in bright light in thin layer)</td>
<td>Melanin</td>
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<td></td>
<td>Quinacrine*</td>
<td></td>
<td>Methemoglobin</td>
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<td></td>
<td>Nitrofurantoin*</td>
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<td>Myoglobin</td>
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<td></td>
<td>Phenacetin*</td>
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<td>Bile pigments</td>
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<td></td>
<td>Riboflavin (large quantities)*</td>
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<td>Thymol*</td>
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<td></td>
<td>Phenolsulfonphthalein (acidic urine)*</td>
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<td>Phenolic compounds*</td>
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<td></td>
<td></td>
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<td>Nitrites*</td>
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<td>Aniline dyes*</td>
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<td></td>
<td></td>
<td></td>
<td>Homogentisic acid*</td>
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<tr>
<td>Blue</td>
<td>Methylene blue</td>
<td>Colorless</td>
<td>Very dilute urine (diuretics, diabetes mellitus, diabetes insipidus, glucocorticoid excess, fluid therapy, overhydration)</td>
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<td></td>
<td>Indigo carmine and indigo blue dye*</td>
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<td>Indicans*</td>
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<td><em>Pseudomonas</em> infection*</td>
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<td>Water-soluble chlorophyll*</td>
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<td>Toluidine blue*</td>
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<td></td>
<td>Triamterene*</td>
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<td>Amitriptyline*</td>
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<td>Anthraquinone*</td>
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<td></td>
<td>Blue food dye*</td>
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<tr>
<td>Green</td>
<td>Methylene blue</td>
<td>Milky white</td>
<td>Lipid</td>
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<td></td>
<td>Dithiazanine</td>
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<td>Pyuria</td>
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<td>Urate crystalluria</td>
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<td></td>
<td>Evan’s blue*</td>
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<td>Bilirubin</td>
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<td>Biliverdin</td>
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<td>Anthraquinone*</td>
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<td></td>
<td>Green food dye*</td>
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<tr>
<td>Red, pink, red-brown, red-orange, or orange</td>
<td>Hematuria</td>
<td>Brown</td>
<td>Methemoglobin</td>
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<tr>
<td></td>
<td>Hemoglobinuria</td>
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<td>Melanin</td>
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<td></td>
<td>Myoglobinuria</td>
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<td>Sulfasalazine*</td>
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<td>Porphyrinuria</td>
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<td>Congo red</td>
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<td>Neoprontosil</td>
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<td>Sulfonamides*</td>
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<td>Warfarin (orange)*</td>
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<td>Bismuth*</td>
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<td>Food pigments (rhubarb, beets, blackberries)*</td>
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<td>Mercury*</td>
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<td>Carbon tetrachloride*</td>
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<td>Feces (rectal-urinary fistula)</td>
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<td>Fava beans*</td>
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<td>Phenazopyridine</td>
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<td>Phenothiazine*</td>
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<td>Diphenylhydantoin*</td>
<td>Metronidazole*</td>
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<td>Bromsulphalein</td>
<td>Methocarbamol*</td>
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<td>Chronic heavy metal poisoning*</td>
<td>Anthracin cathartics*</td>
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<td>Rifampin*</td>
<td>Clofazimine*</td>
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<td>Eosin*</td>
<td>Furazolidone*</td>
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<td>Rifabutin*</td>
<td>Copper toxicity</td>
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<td>Acetazolamide*</td>
<td>Red food dye*</td>
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<td>Orange-yellow</td>
<td>Highly concentrated urine</td>
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<td>Excess urobilin</td>
<td>Bilirubin</td>
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<td>Fluorescein sodium*</td>
<td>Flutamide*</td>
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<td>2,4-d*</td>
<td>Acetazolamide*</td>
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<tr>
<td>Orange food dye*</td>
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*Only observed in human beings

- **Turbidity**
  - Urine is typically clear
  - May be less transparent with pigmenturia, crystalluria, hematuria, pyuria, lipiduria, or other compounds such as mucous.
  - The increased turbidity may disappear with centrifugation of the sample depending on the cause of increased turbidity.

- **Odor**
  - Normal urine has a slight odor of ammonia; however, the odor is dependent on urine concentration.
  - Some species, such as cats (felinine) and goats, have pungent urine odor because of urine composition.
  - Bacterial infection may result in a very strong odor due to pyuria and a strong ammonia odor if the bacterial organism produces urease.

- **Centrifuge** for 3-5 minutes at 1500-2000 rpm
  - Pour off supernatant
  - Can do USG
  - Use for dipstick (semi-quantitative chemical testing)
  - Leave small pellet with little urine in tube for microscopic examination

- **Dipstick (semi-quantitative, colorimetric reagent strips)**
  - Reagent strips such as Multistix® or Chemstrip® can be used to perform several semi-quantitative chemical evaluations simultaneously.
  - Determine urine pH, protein glucose, ketones, bilirubin/urobilinogen, and occult blood.
  - Some reagent strips include test pads for leukocyte esterase (for detection of white blood cells, (WBC)), nitrite (for detection of bacteria), and USG; these are not valid in animals and should not be used.
• Reagent strips are adversely affected by moisture and have a limited shelf life. Bottles should be kept tightly capped, and unused strips should be discarded after their expiration date.

<table>
<thead>
<tr>
<th>pH</th>
<th>Varies from 5.0-9.0</th>
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<tbody>
<tr>
<td></td>
<td><strong>Accurate to within 0.5 pH units</strong></td>
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<td></td>
<td>A reading of 6.5 means the actual pH is likely to be between 6.0 and 7.0</td>
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<td></td>
<td>Typically acidic in dogs and cats and alkaline in horses and ruminants,</td>
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<td></td>
<td>Variable depending on diet, medications, or presence of disease.</td>
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<td>Falsely increased if left at room temperature</td>
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<td></td>
<td>A bacterial urinary tract infection with a urease-producing microbe will result in alkaluria.</td>
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<tr>
<td></td>
<td>Urine pH will affect crystalluria because some crystals, such as struvite, form in alkaline urine, while other crystals, such as cystine, form in acidic urine.</td>
</tr>
</tbody>
</table>

• **Protein**

  • **Methods**
  
  • **Dipstick**
  
  • Detects primarily albumin
  
  • 30-3,000 mg/dl
  
  • False negative for other proteins (e.g. globulins)
  
  • False positive with alkaluria
  
  • Sulfasalicylic acid precipitation test
  
  • Detects albumin and globulins
  
  • Used as verification
  
  • Not accurate
  
  • **Quantitative**
  
  • Measure albumin or protein in a 24-hour urine sample
  
  • **Urine protein to urine creatinine (UPC)**
  
  • < 0.2 = normal
  
  • 0.2 – 0.4 (cats) and 0.5 (dogs) = borderline
  
  • > 0.4 (cats) and > 0.5 (dogs) = abnormal
  
  • **Microalbumin**
  
  • Semi-quantitative ELISA test for microalbuminuria
  
  • 1-30 mg/dl
  
  • Different kits for dogs and cats

• Interpretation
• Small amount is normal
• Alkaluria can give slight false positive reaction
• Can be seen with inflammation, hemorrhage, or glomerular disease.
• A positive reaction must be interpreted in light of USG, pH, and urine sediment examination.
  • For example, a trace amount of protein in concentrated urine is less significant than a trace amount of protein in dilute urine.
  • Presence of other proteins, such as Bence-Jones proteins, will give false negative results.
• Glucose
  • Not present normally
  • Renal threshold is >180 mg/dl in most species; >240 mg/dl in cats.
  • With euglycemia, the amount of filtered glucose is less than the renal threshold and all of the filtered glucose is reabsorbed in the proximal renal tubules.
  • Glucosuria can result from hyperglycemia (due to diabetes mellitus, excessive endogenous or exogenous glucocorticoids, or stress) or from a proximal renal tubular defect (such as primary renal glucosuria or Fanconi syndrome).
  • If glucosuria is present, blood glucose concentration should be determined.
• Ketones
  • Detects acetate and acetoacetate, but not beta-hydroxybutyrate.
  • Associated with either primary ketosis (ruminants), ketosis secondary to diabetes mellitus (small animals), and occasionally with prolonged fasting or starvation.
  • A false positive reaction can occur with presence of reducing substances in urine.
• Bilirubin / urobilinogen
  • Bilirubinuria occurs when conjugated bilirubin exceeds renal threshold as with liver disease or hemolysis.
    • In dogs with concentrated urine, a small amount of bilirubin can be normal.
    • Bilirubinuria is ALWAYS abnormal in cats
    • A tablet test, Ictotest®, is more sensitive
    • Pigmenturia may result in a false positive reaction.
  • Urobilinogen, formed from bilirubin by intestinal microflora, is absorbed into the portal circulation and is excreted renally
    • Not specific enough to be clinically useful.
• Occult blood
  • A "pseudoperoxidase" method to detect intact red blood cells (RBC), hemoglobin, and myoglobin.
  • A positive reaction can be due to hemorrhage (hematuria), intravascular hemolysis (hemoglobinuria), or myoglobinuria.
    • The latter two processes can be distinguished by examination of plasma; plasma will appear pink to red after intravascular hemolysis, while myoglobin is rapidly cleared from plasma resulting in clear plasma.
    • As with other colorimetric test pads, discolored urine may yield false positive results.
    • A positive result should be interpreted with microscopic examination of urine sediment.
• Other semi-quantitative test pads
  • Nitrite, leukocyte, and urine specific gravity – there are test pads for detection of nitrite (from bacterial infection), leukocytes (leukocyte esterase from white blood cells present in urine), and urine specific gravity. These are not reliable for use in animals.
• **Urine specific gravity**
  - Indirect measure of osmolality, which is a better measure of concentration
    - Do on whole urine or supernatant after centrifugation if urine is discolored
  - Determined using a refractometer designed for veterinary samples, which includes a scale calibrated specifically for cat urine.
    - USG for species other than cats should be determined using the scale for dogs
    - Interface of dark and light
    - Mass relative to the mass of deionized water (USG = 1.000)
  - Highly variable, depending on fluid and electrolyte balance of the body
    - Interpretation depends on clinical presentation and serum chemistry findings.
    - An animal that is dehydrated or has other causes of prerenal azotemia will have hypersthenuric urine with a USG >1.025-1.040 (depending on species).
    - Dilute urine in a dehydrated or azotemic animal is abnormal and could be caused by renal failure, hypoadrenocorticism, hyperadrenocorticism, hypercalcemia, diabetes mellitus, hyperthyroidism, diuretic therapy, or diabetes insipidus.
    - Glucosuria increases the refractive index of urine resulting in an increased USG despite increased urine volume.

• **Urine Sediment**
  - Following centrifugation, decant supernatant leaving approximately 0.5 ml of urine and sediment in the tip of the conical tube.
  - Re-suspend pellet by tapping the tip of the conical tube against the table several times.
  - Transfer a few drops of the sediment to a glass slide, and a cover slip is applied.
  - Examination of unstained urine is recommended for routine samples.
  - Microscopic examination is performed at 100X (for crystals, casts, and cells) and 400X (for cells and bacteria) magnifications.
    - Contrast of the sample is enhanced by closing the iris diaphragm and lowering the condenser of the microscope.
  - Stains such as Sedistain® and new methylene blue can be used to aid in cell identification but tend to dilute the specimen and introduce artifacts such as stain precipitate and crystals.
  - Interpret results with urine dipstick evaluation, USG, and urine specimen handling

• **Cells**
  - **Red blood cells**
    - Small and round and have a slight orange tint and smooth appearance.
    - Normal urine should contain <5 RBC/field at 400X magnification.
    - Increased RBC in urine (hematuria) indicates hemorrhage somewhere in the urogenital system; however, sample collection by cystocentesis or catheterization may induce hemorrhage.
  - **White blood cells**
    - Slightly larger than RBC and have grainy cytoplasm.
    - Normal urine should contain <5 WBC/field at 400X magnification.
    - Increased WBC (pyuria) can occur due to inflammation, infection, trauma, or neoplasia.
    - Catheterization or collection of voided urine may introduce a few WBC from the urogenital tract.
White blood cell (left) and 2 red blood cells (right)

- **Epithelial cells**
  - Transitional epithelial cells, a common urine contaminant derived from the bladder and proximal urethra, resemble WBC but are larger.
  - They have a greater amount of grainy cytoplasm and a round, centrally located nucleus.
  - In a voided urine sample, squamous epithelial cells may be observed.
  - They are large, oval to cuboidal in shape, and may or may not contain a nucleus.
  - Occasionally, neoplastic transitional cells may be observed in an animal with a transitional cell carcinoma or neoplastic squamous cells may be observed in an animal with a squamous cell carcinoma.

- **Cylindruria (casts)**
  - Elongated, cylindrical structures formed by mucoprotein congealing within renal tubules and may or may not contain cells.
  - **Hyaline casts** have parallel sides and rounded ends, and are composed of mucoprotein.
    - They may occur with fever, exercise, and renal disease.
  - **Cellular casts**
    - **Epithelial cellular casts** form from entrapment of sloughed tubular epithelial cells in the mucoprotein; they may be observed with renal disease.
    - **Granular casts** are thought to represent degenerated epithelial cellular casts.
      - Most common type of cast
    - **Waxy casts** have a granular appearance, and are thought to arise from long-standing granular casts.
      - They typically have sharp borders with broken ends.
• A few hyaline or granular casts are considered normal. However, presence of cellular casts or other casts in high numbers indicate renal damage, and may be one of the earliest laboratory abnormalities noted with toxic damage to renal epithelial cells (eg, gentamicin, amphotericin B).
  • **Erythrocyte casts** form because of renal hemorrhage.
  • **WBC casts** occur because of renal inflammation, as with pyelonephritis.
• **Fatty casts** are not common, but can be observed with disorders of lipid metabolism, such as diabetes mellitus.

• **Infectious organisms**
  • Presence of **bacteria** in urine collected by cystocentesis indicates infection.
    • Small numbers of bacteria from the lower urogenital tract may contaminate voided samples or samples collected by catheterization, and do not indicate infection.
    • Bacterial rods are most easily identified in urine sediment.
    • Particles of debris may be mistaken for bacteria.
    • Suspected bacteria can be confirmed by staining urine sediment with Gram’s stain; however, aerobic culture is best to confirm a bacterial urinary tract infection.
  • Rarely, **yeast** and **fungal hyphae** and **parasitic ova** may be observed in urine sediment.
    • Their presence is not always associated with clinical disease.
    • Parasitic ova observed include *Stephanus dentatus, Capillaria plica, Capillaria felis,* and *Dioctophyma renale.*
    • Additionally, microfilariae of *Dirofilaria immitis* may be observed in urine sediment.
• **Crystals**
  - Many urine sediments contain crystals.
  - The type of crystal present depends on urine pH, concentration of crystallogenic materials, urine temperature, and length of time between urine collection and examination.
  - Crystalluria is not synonymous with urolithiasis, and is not necessarily pathologic.
  - Furthermore, uroliths may form without observed crystalluria.

• **Struvite**
  - Struvite crystals appear typically as “coffin-lids” or “prisms”; however, they may be amorphous in appearance.
    - Occur in alkaline pH
  - Struvite crystals are commonly observed in canine and feline urine.
    - Herbivores often have struvite crystalluria due to alkaluria
  - Struvite crystalluria in dogs is not a problem unless there is a concurrent bacterial urinary tract infection with a urease-producing microbe.
    - Without an infection, struvite crystals in dogs will not be associated with struvite urolith formation.
  - Some animals, such as cats, however, do form struvite uroliths without a bacterial urinary tract infection.
    - In these animals, struvite crystalluria may be pathologic.

• **Calcium oxalate**
  - Occurs in acidic pH
  - Two forms:
    - Monohydrate: “dumb-bell” in shape.
    - Dihydrate: “envelope” or “square with X”
  - Occurs less commonly in dogs and cats
    - An unusual form of calcium oxalate crystals is typically seen in association with ethylene glycol toxicity.
    - May be associated calcium oxalate urolith formation.
  - Common in horses and cattle

• **Calcium carbonate**
  - Common in horses, rabbits, guinea pigs, and goats
  - Occurs in alkaline pH
• Variably sized and often appear as large spheroids with radial striations
  • May also be smaller crystals with round, ovoid, or dumbbell shapes
  • Colorless to yellow-brown

• **Ammonium urate**
  • Occur in acidic pH
  • Yellow-brown spheres with irregular, spiny projections; however, they may also be amorphous
  • Ammonium acid urate crystals suggest liver disease (eg, portosystemic shunt).
  • Certain species, such as birds and reptiles, and certain breeds of dogs, specifically Dalmatians, can normally have ammonium acid urate crystalluria.

• **Cystine**
  • Cystine crystals are six-sided and of variable size.
  • They occur in acidic urine.
  • Presence of cystine crystals represents a proximal tubular defect in amino acid reabsorption.
  • Cystinuria has been reported to occur in many breeds of dogs and rarely in cats, but Dachshunds, Newfoundlands, English bulldogs, and Scottish terriers have a high incidence of cystine urolithiasis.

• **Bilirubin**
  • Bilirubin crystals occur with bilirubinuria; however, these may be normal in small numbers in dogs.

- **Lipid**
  • Fat droplets are commonly present in urine from dogs and cats and may be mistaken for RBC. However, they often vary in size and tend to float on a different plane of focus than the remainder of the sediment. They are not considered to be pathologic.

• **Spermatozoa**
  • Spermatozoa may be observed normally in urine collected from male dogs.

• **Artifact**
  • Occasionally, plant material may be observed in urine samples collected by voiding. When present, they indicate contamination of the urine sample, and are not pathologic.
• Talc powder granules may be observed in voided samples if the collector is wearing gloves

• **Enzymuria.**
  • Enzymatic activity, used as a marker for AKI, belong to enzymes that are found within the renal tubular cells.
  • These enzymes are too large to be filtered through a normal glomerulus, and so in the absence of profound glomerular disease, a rise in the urinary activity of such enzymes is typically caused by acute damage to the tubules and leakage from the tubular cells.
  • Urinary enzymes, GGT, and NAG are the most commonly used and most practical enzymes to assess urinary activity.
    • NAG is found within the proximal tubular lysosomes and GGT with the proximal tubule brush border.
    • The activity of these enzymes is a sensitive method of detecting acute tubular kidney injury, more sensitive than changes in glomerular filtration rate, serum biochemistry (azotemia) and clinical signs.
    • Changes in urinary enzyme concentrations, GGT and NAG, can be estimated by enzyme to creatinine ratios on spot urine samples, deeming 24-hour urine collections not absolutely necessary.
    • Examples of such cases include the use of renal toxic chemotherapeutic agents, the use of aminoglycosides, a very recent overdose of a non-steroidal anti-inflammatory drug (NSAID) or the use of an NSAID in a renal compromised patient.

• **Point-of-care testing for urinary tract infections**
  • These tests may detect presence of bacteria, determine type of bacteria (Gram + versus Gram -), identify the bacterial organism, and/or provide susceptibility to a limited number of antimicrobial agents

• **Other infectious agents**
  • Urine may be submitted for Leptospirosis PCR for diagnosis of leptospirosis or for fungal PCR for diagnosis of fungal infections such as Blastomycosis, Aspergillus, and Histoplasmosis

• **Bladder tumor antigen test (VBTA)**
  • The VBTA can be used as a screening test for transitional cell carcinoma in dogs.
  • The results are not specific and non-neoplastic disease (e.g. urinary tract infections, hematuria, etc) can give positive results.
  • A negative test; however, is meaningful in that a transitional cell carcinoma is not likely to be present.
  • This test may be useful for routine screening of dogs at higher risk of developing transitional cell carcinoma (e.g. Scottish terriers) that do not have other signs or laboratory findings of lower urinary tract disease.