Urine analysis

By
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Functions of Renal system

Excretory functions	
Metabolic waste	Urea , Creatinin
Drug clearance	
Toxin clearance	

Regulatory functions	
Water balance	Blood / urine volume change
Electrolyte balance	Na^{+}, K^{+}, Ca^{++}, Cl^{-}, $HCO3^{-}$
Acid base balance	

Functions of Renal system

Endocrine functions	
Renin	Works with angiotensin system
Prostaglandins	Works on renal blood flow
Erythropoitin	Synthesis of new RBC

Metabolic functions	
Vitamin D synthesis	Works in association with para-thyroid hormone

Urine collection

Table 3–4 Types of Urine Specimens

Type of Specimen	Purpose
Random	Routine screening
First morning	Routine screening Pregnancy tests Orthostatic protein
Fasting (second morning)	Diabetic screening/monitoring
2-hour postprandial	Diabetic monitoring
Glucose tolerance test	Optional with blood samples in glucose tolerance test
24-h (or timed)	Quantitative chemical tests
Catheterized	Bacterial culture
Midstream clean-catch	Routine screening Bacterial culture
Suprapubic aspiration	Bladder urine for bacterial culture Cytology
Three-glass collection	Prostatic infection

Urine analysis & body fluids
- Susan king
-Marjorie Schaub

Urine collection & preservation

Table 3–3 Urine Preservatives

Preservatives	Advantages	Disadvantages	Additional Information
Refrigeration	Does not interfere with chemical tests	Raises specific gravity by hydrometer Precipitates amorphous phosphates and urates	Prevents bacterial growth 24 h[3]
Thymol	Preserves glucose and sediments well	Interferes with acid precipitation tests for protein	
Boric acid	Preserves protein and formed elements well Does not interfere with routine analyses other than pH	May precipitate crystals when used in large amounts	Keeps pH at about 6.0 Is bacteriostatic (not bactericidal) at 18 g/L; can use for culture transport[4] Interferes with drug and hormone analyses
Formalin (formaldehyde)	Excellent sediment preservative	Acts as a reducing agent, interfering with chemical tests for glucose, blood, leukocyte esterase, and copper reduction	Rinse specimen container with formalin to preserve cells and casts
Toluene	Does not interfere with routine tests	Floats on surface of specimens and clings to pipettes and testing materials	
Sodium fluoride	Prevents glycolysis Is a good preservative for drug analyses	Inhibits reagent strip tests for glucose, blood, and leukocytes	May use sodium benzoate instead of fluoride for reagent strip testing[5]

Urine collection & preservation

Table 3–2 Changes in Unpreserved Urine

Analyte	Change	Cause
Color	Modified/darkened	Oxidation or reduction of metabolites
Clarity	Decreased	Bacterial growth and precipitation of amorphous material
Odor	Increased	Bacterial multiplication or breakdown of urea to ammonia
pH	Increased	Breakdown of urea to ammonia by urease-producing bacteria/ loss of CO_2
Glucose	Decreased	Glycolysis and bacterial use
Ketones	Decreased	Volatilization and bacterial metabolism
Bilirubin	Decreased	Exposure to light/photo oxidation to biliverdin
Urobilinogen	Decreased	Oxidation to urobilin
Nitrite	Increased	Multiplication of nitrate-reducing bacteria
Red and white blood cells and casts	Decreased	Disintegration in dilute alkaline urine
Bacteria	Increased	Multiplication

Urine collection & preservation

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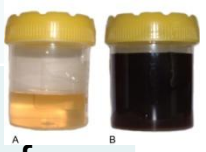
Urine – Routine analysis

Routine analysis Includes the examination of physical and chemical characteristics and microscopic studies of cellular & non cellular components

Physical examination	**Color :**
Appearance	Normal urine color has wide range from pale yellow, straw, light yellow, dark yellow amber because of the presence of urochrome pigment and some urobilin and uroerythrin
	The color is effected by • Concentration • pH • Metabolic activity • Drugs • Diet (beetroot)

Urine – Routine analysis

Physical examination - Abnormalities in color	
Colorless to pale yellow • High intake of water • Reduced sweat • Using diuretics • Diabetes Mellitus • Diabetes Insipidus • Alcohol intake • Nervousness	**Dark yellow, Amber, orange** • low water intake • excessive sweating • dehydration (high grade fever / burns) • Diet (carrots , viatamin A supplements) • some drugs
Brownish yellow • Bilirubin – yellow foam upon shaking • Urobilin – no yellow foam upon shaking	**Pink – red** • Presence of blood – UTI infection, Trauma…. **Blue – green** Infection - Pesudomonas
Yellow - green • Bilirubin upon oxidation turns green	**Black urine** • Alkaptonurea – inborn error of metabolism (tyrosine metabolism



Urine – Routine analysis

Physical examination	Abnormalities in Odor
	Normal urine has faint aromatic odor
	Fruity odor – Diabetic urine – acetone
	Offensive odor – infection
	Apple odor – diet (asparagus)

Urine – Routine analysis

Physical examination - Abnormalities in Volume

Normal adult urine volume – 600 ml – 2400 ml
Normal children volume – 200 ml – 400 ml / day
Depends on water intake, ambiance temperature, mental and physical state, use of drugs.

Oligourea : decrease in urine less than 400 ml

- low water intake
- Dehydration – vomiting, Diarrhea, sweating
- Renal Ischemia – heart failure, Hypotension
- Obsrtuction by – stone, tumor…

Polyurea : increase in urine more than 2500 ml

- Increased fluid intake,
- Increased salt and protein diet,
- Use of diuretics (some drugs, green tea)
- Intravenous saline
- Diabetes
- Renal diseases

Urine – Routine analysis

Physical examination	Abnormalities in Volume
	Anuria : complete stoppage of urine flow • Presence of stone or tumor • Severe renal defect and loss of urine formation • Hemolytic reaction (after blood transfuction)
	Nocturia : excessive urination in night

Physical examination	Specific gravity	
	Indicates kidneys ability to concentrate / dilute Normally it is 1.002 – 1.035	
	Low specific gravity • Diabetes Insipidus • Glomerulonephritis • Renal damage	**High specific gravity** • Diabetes mellitus • High fever • drugs…

Urine – Routine analysis

Chemical examination - pH	
One of the regulatory functions of kidneys Normal pH range is 4.6 – 8.0 If urine pH is moret han 9 sample can be rejected	
Acidic urine • High protein diet • Starvation • dehydration • Some drugs	**Alkaline urine** • High veg. diet • Urinary tract infections • Some drugs
• Crystals of Ca. oxalate, uric acid, cystine…	• Crystals of Ca. carbonate, Ca. phosphate, Mg. Phosphate….

Urine – Routine analysis

Chemical examination - Protein

Small amount of protein appears daily in normal urine (50 – 150 mg / Day). More than 150 mg/day can be called proteinuria

Proteinurea

- Glomerular membrane damage
- Absorption problems
- Heavy metal poisoning
- Some cancers

Non pathological proteinurea

- High grade fever
- Emotional disturbances
- Later months of pregnancy
- excessive excercises

Urine – Routine analysis

Chemical examination - Glucose

Normally all the glucose filtered by glomerulus is reabsorbed.
If blood glucose concentration is increased, reabsorption of glucose is decreased and glucose appears in urine
Glycosuria – excess sugar in urines
Threshold of glucose is – 180 mg / dl

Glycosuria

- Reabsorption defects
- Diabetes
- Alimentary glycosuria
- Stress
- Diseases of pancreas

Urine – Routine analysis

Chemical examination – Blood, hemoglobin and myoglobin

Normally there is no blood or hemoglobin in urine. If present it is called
Hematuria – Presence of RBC
Hemoglobinuria – Presence of free hemoglobin
Myoglobinuria – Presence of myoglobin which is heme, muscle protein

Hematuria
- Presence of RBC
- Renal disease
- Renal Calculi
- Tumor
- Trauma
- lower UTI infections
- Catheterization
- **Bleeding disorders**
- Leukemia
- Hemophelia

Hemoglobinuria
- Hemolytic anemia
- Severe burns
- Transfusion reaction
- Poisoning
- Severe exercise
- Some infections

Myoglobinuria
- Muscular trauma
- Convulsions
- Coma
- Muscle diseases

Urine – Routine analysis

Chemical examination – Ketone bodies

Ketones are products of fat metabolism
Acetone, Acetoacetic acid and beta-hydroxybutyric acid are examples

Ketonurea occurs in

- Diabetes
- Starvation

Urine – Routine analysis

Urine – Routine analysis

Microscopic examination of urine - techniques

Table 6–5 Urinalysis Microscopic Techniques

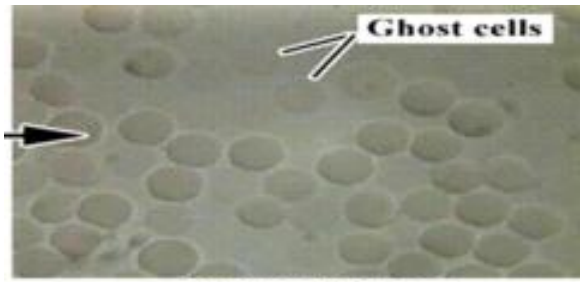
Technique	Function
Bright-field microscopy	Used for routine urinalysis
Phase-contrast microscopy	Enhances visualization of elements with low refractive indices, such as hyaline casts, mixed cellular casts, mucous threads, and *Trichomonas*
Polarizing microscopy	Aids in identification of cholesterol in oval fat bodies, fatty casts, and crystals
Dark-field microscopy	Aids in identification of *Treponema pallidum*
Fluorescence microscopy	Allows visualization of naturally fluorescent microorganisms or those stained by a fluorescent dye
Interference-contrast	Produces a three-dimensional microscopy-image and layer-by-layer imaging of a specimen

Urine – Routine analysis

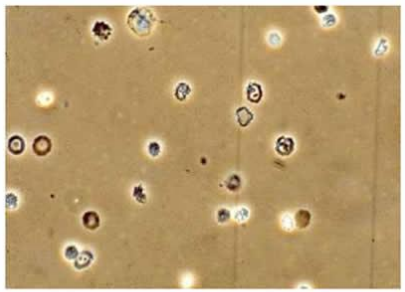
Microscopic examination of urine
Normal urine contains small number of cells and other elements Epithelial cells from nephron, pelvis, bladder and urethra
Spermatozoa from prostate, some RBC, WBC and occasional casts also found normally
Method of examination
Sedimentation by centrifugation
Resuspend the sediment and pour on glass slide and cover slip
Examination
At low magnification number of casts cells can be reported (5-10 casts/LPF)
At high magnification crystals, cells, bacteria can be reported (1-5 wbc / HPF)

Urine – Routine analysis

Microscopic examination of urine - RBC
Theoretically no RBLC should be found. But some found in healthy individual also
If 1 or more RBC found in every HPF the specimen is abnormal
Shape of RBC might be effected by urine concentration
Ghost cell (erythrocyte cell membrane)
Faint RBC which is exposed to hemolysis due hypotonic alkaline urine Indicates presence of hemoglobin
Dysmorphic cell (shrinking RBC)
Possible hemorrhage or trauma
Indicates hypertonic urine

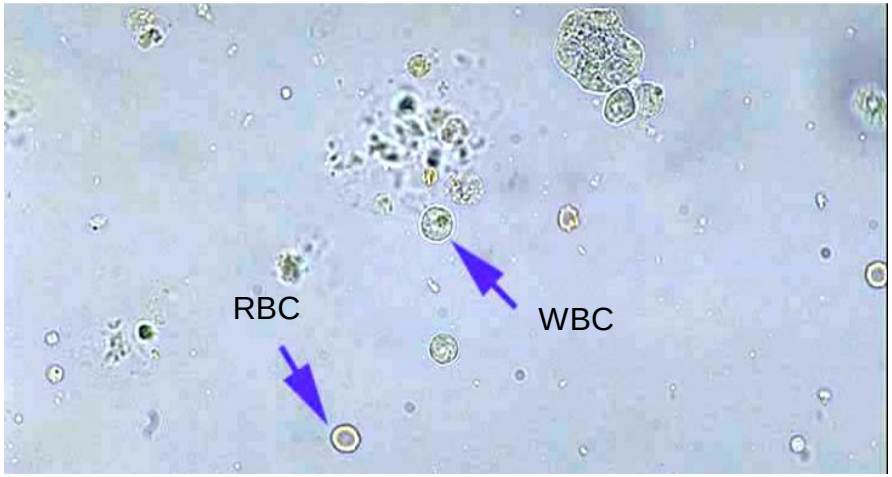


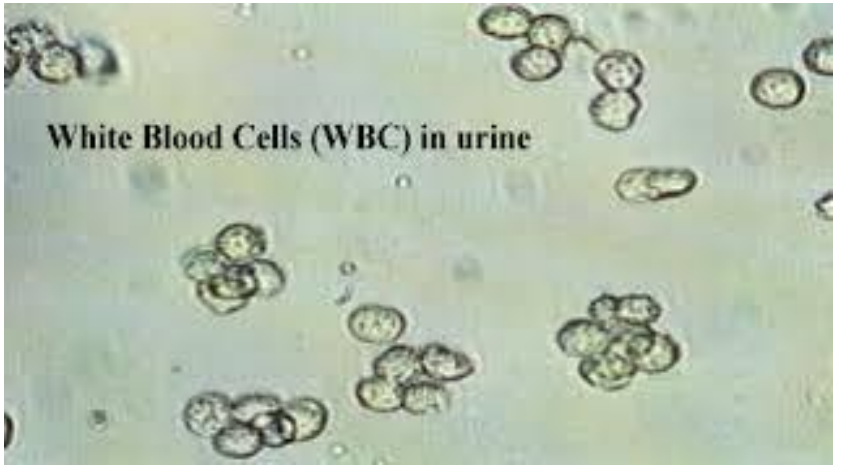
Swollen RBC's and Ghost (Lysed) Cells


GLOMERULAR OR DYSMORPHIC ERYTHROCYTES

Urine – Routine analysis

Microscopic examination of urine - WBC
Presence of abnormal number of WBC in urine is referred as **Pyuria**
Appears in infection and inflammation in upper or lower urinary tract Also found during acute glomerulonephritis
Origin of WBC in urine
• Through glomerular damage • Migration through the site of infection
They are granular in appearance





Urine – Routine analysis

Microscopic examination of urine – RBC & WBC

Summary of Microscopic RBCs

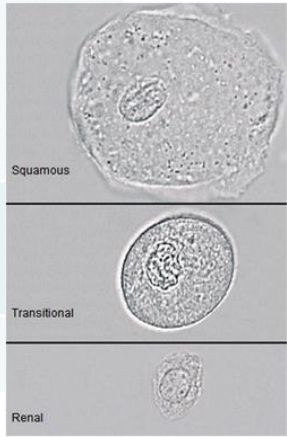
Appearance:	Non-nucleated biconcave disks Crenated in hypertonic urine Ghost cells in hypotonic urine Dysmorphic with glomerular membrane damage
Sources of	Yeast cells
Identification	Oil droplets
error:	Air bubbles
Reporting:	Average number per 10 hpfs
Complete urinalysis correlations:	Color Reagent strip blood reaction

Summary of Microscopic WBCs

Appearance:	Larger than RBCs Granulated, multilobed neutrophils Glitter cells in hypotonic urine Mononuclear cells with abundant cytoplasm
Sources of identification error:	Renal tubular epithelial cells
Reporting:	Average number per 10 hpfs
Complete urinalysis correlations:	Leukocyte esterase Nitrite Specific gravity pH

Urine – Routine analysis

Microscopic examination of urine – Epithelial cells		
Normally few epithelial cells are present because of cell turnover which may originate from any site of urogenitory tract		
Marked increase may indicate inflammation		
Types of epithelial cells		
Renal Tubular	**Transitional**	**Squamous**
Most common	Originate from bladder and urethra	Originate from genitals and lower urethra
Found during tubular necrosis by viral infections / toxins	Generally not associated with pathology unless Abnormal number and morphology	Most frequently seen and least significant
Play role in graft rejection		



Urine – Routine analysis

Summary of Epithelial Cells

Squamous Cells

Appearance:	Largest cells in the sediment with abundant, irregular cytoplasm and prominent nucleii
Sources of error:	Rarely encountered, folded cells may resemble casts
Reporting:	Rare, few, moderate, or many per lpf
Complete urinalysis correlations:	Clarity

Transitional Cells

Appearance:	Spherical, polyhedral, or caudate with centrally located nucleus
Sources of error:	Spherical forms resemble RTE cells
Reporting:	Rare, few, moderate, or many per hpf
Complete urinalysis correlations:	Clarity; blood, if malignancy-associated

RTE Cells

Appearance:	Rectangular, columnar, round, oval or, cuboidal with an eccentric nucleus possibly bilirubin-stained or hemosiderin-laden
Sources of error:	Spherical transitional cells Granular casts
Reporting:	Average number per 10 hpfs
Complete urinalysis correlations:	Leukocyte esterase and nitrite (pyelonephritis) Color Clarity Protein Bilirubin (hepatitis) Blood

Oval Fat Bodies

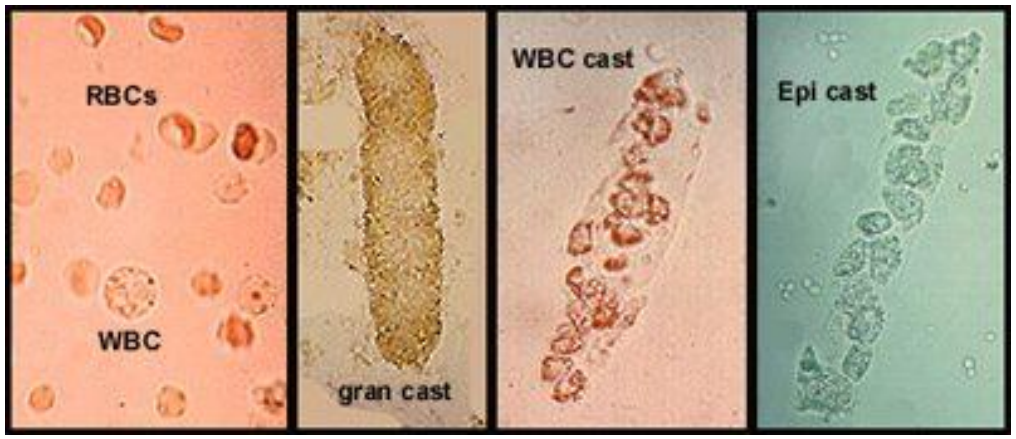
Appearance:	Highly refractile RTE cells
Sources of error:	Confirm with fat stains and polarized microscopy
Reporting:	Average number per hpf
Complete urinalysis correlations:	Clarity Blood Protein Free fat droplets/fatty casts

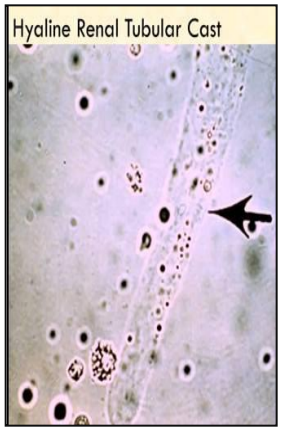
Urine – Routine analysis

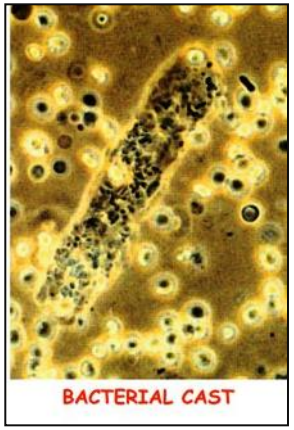
Microscopic examination of urine – Casts		
Small tube shaped particles		
They can be made of WBC, RBC, Kidney cells or substances like protein and fat		
Hyaline casts	**RBC casts**	**Protein casts**
Most common 0-2 /LPF	Not common	Factors responsible are
Contain Tamm-horsfall protein (uromodulin)	Formed of RBC in association with uromodulin	• low flow rate of urine • high salt conc. • low pH
Found during strenuous exercise, fever, dehydration, stress and nephritis, cancer	Generally indicate serious disease like nephritis, renal infraction, SLE…..	All these favor protein denaturation leading to casts like hyaline casts

Urine – Routine analysis

Microscopic examination of urine – Casts	
WBC casts	All found during many pathological conditions
Bacterial casts	
Epithelial cell casts **Granular casts**	
Waxy casts	

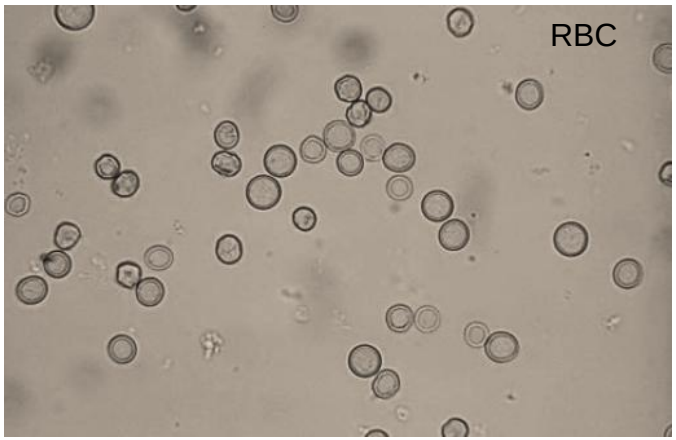






Urine – Routine analysis

Microscopic examination of urine – Microbes
Microbes are usually present because of the normal flora of genitals
Because of their omnipresence nature and rapid multiplying ability, sample collected with extreme caution should be interpreted in view of pathology
Diagnosis generally requires culture
If one organism found more than 100,000 / ml of urine is pathological
If multiple organisms found it might indicate contamination
Yeast often confused with RBC and some crystals, but can be distinguished by their budding



Urine – Routine analysis

Microscopic examination of urine – Crystals
Crystals are generally found in urine and rarely clinical significance
Crystals are formed by precipitation of urine salts. pH, concentration, and temperature effects formation.
pH is the most valuable aid in crystal identification
pH of urine defines the chemicals precipitate
Hence crystals are categorized as normal / abnormal crystals and crystals in acidic / alkaline urine

Urine – Routine analysis

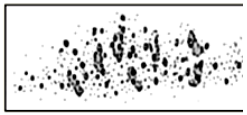
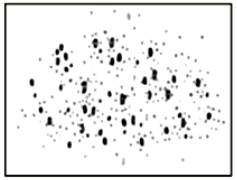
Microscopic examination of urine – Crystals	
1. Normal endogenous crystals	
Acidic urine	**Alkaline urine**
Uric acid plates rhombic, rosettes, wedges and needles. Found during Gout, leukemia	Phosphate are most common crystals found in alkaline urine
Calcium oxalate color less resembles envelopes. Are seen in Genetically susceptible peoples following large doses of Vit. C	Calcium phosphate – colorless thin prisms, plates or needles Ammonium biurate – brownish yellow
	Calcium carbonate – small colorless with bumbell or spherical shapes
2. Abnormal endogenous crystals	
Cystine, cholesterol, leucine, tyrosine, bilirubin, sulfonamide	
Exogenous crystals Starch , talcum powder (from gloves)	

Urine – Routine analysis

Microscopic examination of urine – Crystals

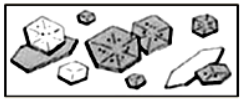
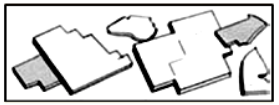
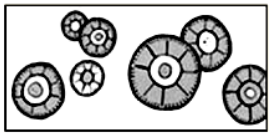
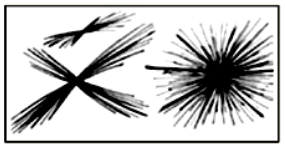
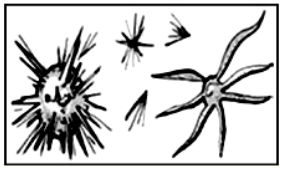
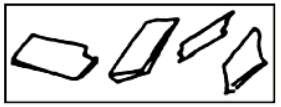
Urine – Routine analysis

Table 6–6 Major Characteristics of Normal Urinary Crystals

Crystal	pH	Color	Solubility	Appearance
Uric acid	Acid	Yellow-brown	Alkali soluble	
Amorphous urates	Acid	Brick dust or yellow brown	Alkali and heat	
Calcium oxalate	Acid/neutral (alkaline)	Colorless (envelopes, oval, dumbbell)	Dilute HCl oval	
Amorphous phosphates	Alkaline Neutral	White–colorless	Dilute acetic acid	
Calcium phosphate	Alkaline Neutral	Colorless	Dilute acetic acid	
Triple phosphate	Alkaline	Colorless ("coffin lids")	Dilute acetic acid	
Ammonium biurate	Alkaline	Yellow-brown ("thorny apples")	Acetic acid with heat	
Calcium carbonate	Alkaline	Colorless (dumbbells)	Gas from acetic acid	

Urine – Routine analysis

Table 6–7 Major Characteristics of Abnormal Urinary Crystals

Crystal	pH	Color	Solubility	Appearance
Cystine	Acid	Colorless	Ammonia, dilute HCl	
Cholesterol	Acid	Colorless (notched plates)	Chloroform	
Leucine	Acid/neutral	Yellow	Hot alkali or alcohol	
Tyrosine	Acid/neutral	Colorless–yellow	Alkali or heat	
Bilirubin	Acid	Yellow	Acetic acid, HCl, NaOH, ether, chloroform	
Sulfonamides	Acid/neutral	Varied	Acetone	
Radiographic dye	Acid	Colorless	10% NaOH	
Ampicillin	Acid/neutral	Colorless	Refrigeration forms bundles	

Next class

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