

Urinalysis Part-I

Physical and Chemical Examination and Diseases

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Urine formation

- Ultrafiltrate
 - Plasma forced by hydrostatic pressure through semipermeable membrane
- 24 hours (average)
 - 170,000 mL filtered plasma
 - 1200 mL urine
 - Less than 1% of what is filtered becomes urine!

Urine composition

- Normally 95% water, 5% solutes
- Composition can be affected by many variables
 - Dietary intake
 - Physical activity
 - Resting metabolic rate
 - Endocrine function



Urine composition - solutes

- Organic
 - Urea
 - 25-35 g /24 hours
 - Creatinine
 - Uric acid
 - Hippuric acid
 - Miscellaneous
 - Carbohydrates
 - Fatty acids
 - Hormones
- Inorganic
 - Sodium chloride
 - 15 g/24 hrs
 - Potassium
 - Sulfate
 - Phosphate
 - Others
 - Ammonium
 - Magnesium
 - Calcium

Urine volume

- Normal daily output 1200-1500 mL
- Variable range (600-2000 mL)
 - Fluid intake
 - Overall hydration (ADH)
 - Non-renal fluid losses
 - Excess solutes



Urine volume

- Oliguria
 - Less than 400 mL/day
 - Dehydration
- Anuria
 - No urine output
 - Renal failure
 - Severe hypotension
- Nocturia
 - Increase in nocturnal urine excretion



Urine volume

- Polyuria
 - Greater than 2500 mL/day
 - Diabetes mellitus (glucose)
 - Diabetes insipidus (ADH)
 - Diuretics
 - Caffeine
 - Alcohol



Quality Assessment

- Preanalytical errors
 - Patient misidentification
 - Wrong test ordered
 - Incorrect specimen type collected
 - Insufficient urine volume
 - Delayed specimen transport
 - Incorrect storage / preservation



Quality Assessment

- Analytical errors
 - Sample misidentification
 - Erroneous instrument calibration
 - Reagent deterioration
 - Poor testing technique
 - Instrument malfunction
 - Interfering substances
 - Misinterpretation of QC data



Quality Assessment

- Post analytical errors
 - Report misidentification
 - Misinterpretation of results
 - Failure to communicate results
 - Failure to document errors



Specimen Collection

- Urine is biohazardous substance
 - Universal Precautions
- Clean, dry, leak-proof containers
 - Routine UA cups
 - Recommended volume 50 mL
 - Sterile cups for cultures
 - 24 hr U/A containers
 - Urine collection bags





Specimen Collection

- Container label
 - Patient's name, ID, date and time of collection
 - Label attached to container, not lid
- Reject and recollect
 - Unlabeled containers, Improper transport
 - Contaminated specimens
 - QNS specimens



Specimen Handling

- Specimens should be delivered within two hours of collection
 - Refrigeration (2-8°C)
 - Inhibits bacterial growth
 - Precipitation of amorphous sediment
 - Return to RT before testing
 - Sterile container
 - Chemical preservative
 - Boric acid general preservative



Types of specimens

- Random
 - Most common
 - Routine screening
- First morning (8-hr)
 - Ideal screening specimen
 - Concentrated sample
 - Pregnancy tests
 - Orthostatic proteinuria
 - Collect immediately after waking



Types of specimens

- 24 hr (timed)
 - Corrects for diurnal variation and effects of daily activity
 - Must begin and end collection period with empty bladder
 - Volume is important measurement
 - Used for quantitative chemical tests
 - Creatinine clearance



Types of specimens

- Catheterized
 - Bacterial culture
- Midstream clean-catch
 - Minimizes bacterial and epithelial contamination
 - Bacterial culture or routine screening
- Pediatric (bag)
 - Non-sterile



Types of specimens

- Suprapubic
 - Needle through abdomen to bladder
 - Bacterial culture
 - Cytology
- Three-glass collection
 - Prostatic infection
 - Not routinely performed



Changes occur in standing urine @ RT

- ↑ in pH due to production of ammonia from urea by bacteria.
- Crystals formation due to precipitation of phosphate and calcium
- ↓ in glucose due to glycolysis
- Oxidation of bilirubin to biliverdin
- Bacterial proliferation
- Cellular elements disintegration

Urine Examination

- Physical examination
- Chemical examination
- Microscopic examination



Physical Examination

Color

- Normal colors
 - Yellow (pale to dark) or Amber
- Normal pigments
 - Urochrome (main pigment)
 - Yellow color
 - Product of endogenous metabolism
 - Gives rough estimate of concentration
 - Uroerythrin
 - Pink color (amorphous sediment)
 - Urobilin
 - Oxidation product of urobilinogen
 - Orange-brown color

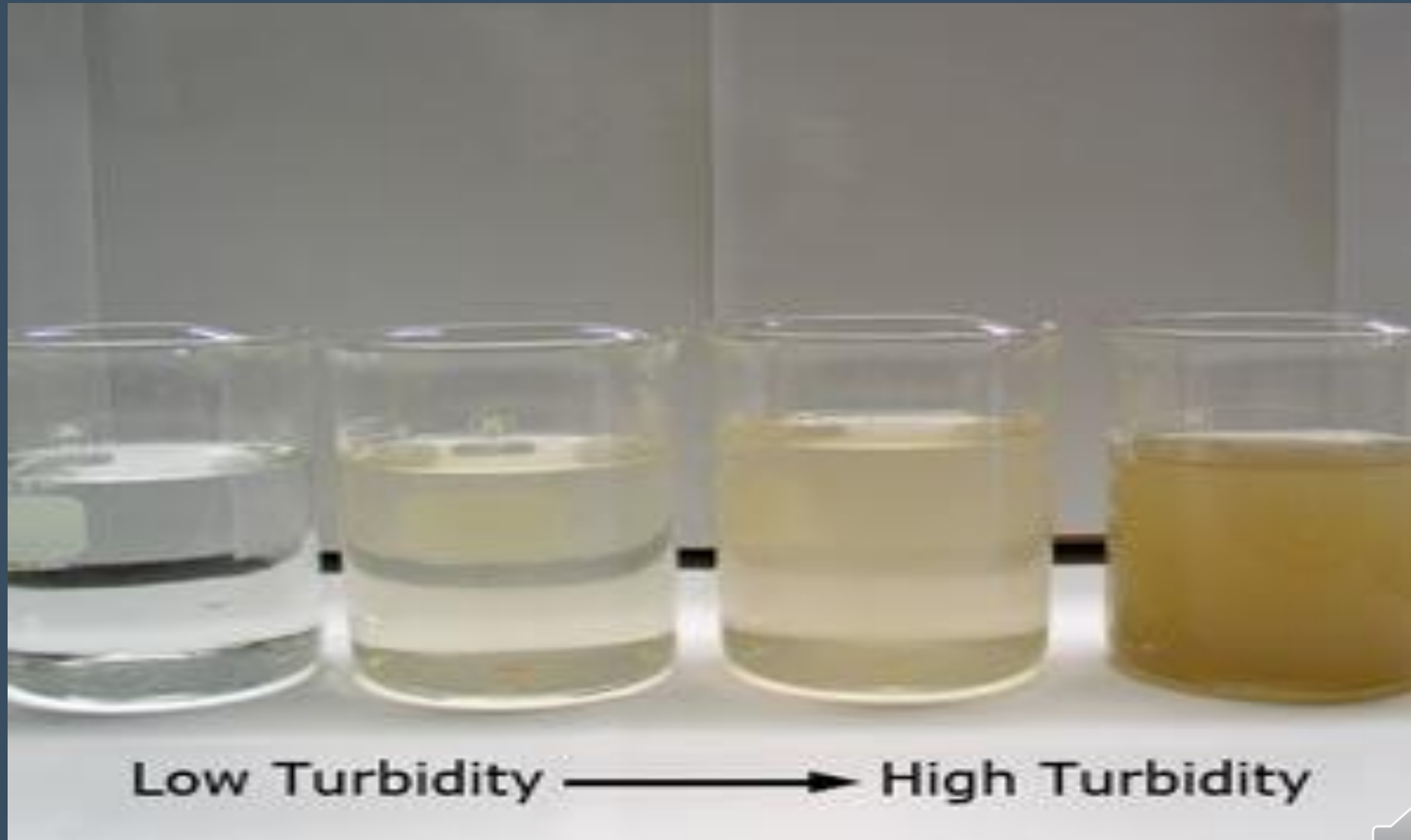


Physical Examination

Color

Color	Conditions
Colorless or pale yellow	Dilute urine (diabetes mellitus, diabetes insipidus, over hydration)
Dark yellow or amber	Normal, concentrated urine , bilirubin
Orange	Bilirubin, medications, foods etc.
Red	Hematuria (cloudy urine), hemoglobinuria (clear urine), myoglobinuria (clear urine) , porphyria , medication, food
Brown or black	Metabolic disorders, medications
Blue to green	Pseudomonas (UTI), Metabolic disorders , medications, foods
Brown (visible RBCs on micro exam)	Oxidation of hemoglobin to methemoglobin

Clarity (turbidity)



Turbidity

Nonpathologic

- Squamous cells
- Mucus
- Amorphous
- Semen
- Talcum powder
- Contrast media

Pathologic

- Non squamous epithelium
- WBCs, RBCs
- Bacteria , yeast
- Abnormal crystals

Odor

- Not part of routine examination
- Aromatic odor in fresh urine due to volatile organic acids
- Ammoniacal odor : Fresh specimen with UTI, old specimen - bacterial decomposition
- Fruity: Ketones
- Maple syrup : Maple syrup urine disease
- Mousy musty: Phenylketonuria
- Foul or fishy: UTI



Specific Gravity (SG)

- Density of a solution compared with the density of distilled water at the same volume and temperature
- Normal SG : 1.003- 1.035
 - Less than 1.003 → not urine
 - Lower than 1.010 → excess fluid volume
 - Greater than 1.035 → deficit fluid volume or Interfering substances



Specific Gravity

- Isosthenuric → SG OF 1.010
 - SG of plasma filtrate entering glomerulus
 - Loss of concentrating ability of tubules seen in end stage renal failure
- Hyposthenuric → less than 1.010
 - Diabetes insipidus, diuretics etc.
- Hypersthenuric → greater than 1.010
 - Diabetes mellitus, glycosuria, dehydration

Chemical examination

Reagent strips

Primary methodology

Chemical-infused absorbent pads attached to plastic strip

Colorimetric reaction with urine

Ancillary tests

Confirmation of strip findings

Interfering substances with strip

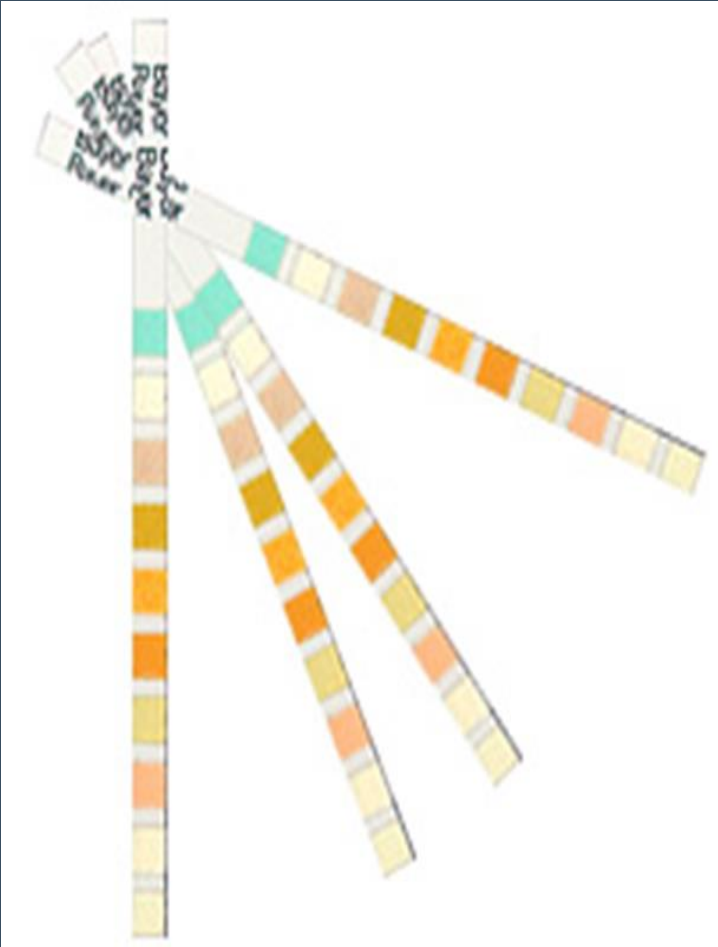


Reagent strips

- Dip into well-mixed specimen and remove excess urine
- Wait the specified length of time before evaluation of findings
 - 30-120 seconds depending on test
- QC strips when opening new bottle and once every 24 hours
 - Negative - all tests negative
 - Positive - +/- one color block of control value



Examined substances



- pH
- Protein
- Glucose
- Ketones
- Blood
- Bilirubin
- Urobilinogen
- Nitrite
- Leukocyte esterase
- Specific gravity





- Normal value ranges from 4.5-8.0
- Double-indicator system
 - Methyl red
 - Red to yellow (pH range 4 to 6)
 - Bromothymol blue
 - Yellow to blue (pH range 6 to 9)
- Sources of error
 - Run over from adjacent pads
 - Old specimens





- Protein error of indicators principle
 - Variation of pH method
 - Independent of the urine pH
 - Indicator contains tetrabromophenol blue within acid buffer (yellow)
 - Albumin accept H^+ from indicator
 - As the indicator loses H^+ , it changes color (green)





- Sources of error
 - False positive
 - Highly buffered alkaline urine
 - Prolonged exposure to urine
 - Certain medications
 - False negative
 - Proteins other than albumin
 - Microalbumin



Sulfosalicylic Acid (SSA)

- Cold precipitation test
- 3 mL of 3% SSA to 3 mL of urine
- Mix by inversion and grade turbidity
 - Turbidity compared to known standards
 - Semiquantitative analysis



Clinical Significance of Urine Protein

- **Prerenal proteinuria**
 - Not indicative of renal disease
 - Causes
 - Free hemoglobin or myoglobin
 - Acute phase reactants
 - Bence Jones protein
 - Monoclonal Ig light chains (myeloma)

Renal proteinuria

- Glomerular damage
 - Immune complex disorders
 - Lupus nephritis, streptococcal GN
 - Diabetes mellitus, amyloidosis
 - Toxic agents and hypertension
- Tubular dysfunction
 - Toxic agents, severe viral infections
 - Fanconi syndrome



Renal proteinuria

- Orthostasic
 - First morning urine negative
 - Later specimens are positive
- Microalbumin
 - Early indicator of diabetic or hypertensive renal disease
 - Not detected by protein indicator



Postrenal proteinuria

- Protein from lower urinary tract
 - Bacterial and fungal infection
 - Inflammatory conditions
 - Semen
 - Vaginal secretions
 - Menses



Glomerular Disorders

Glomerulonephritis

- An overactive immune system may attack the kidney, causing inflammation and some damage.
- Presence of blood, protein and cast in the urine
- Acute > chronic > nephrotic syndrome > renal failure



Glomerular Disorders

Acute Glomerulonephritis

- Damage to glomerular membrane
- Typical urinalysis findings
 - Proteinuria, oliguria, hematuria and dysmorphic RBCs
 - Varied casts - RBC, WBC, hyaline and granular
 - Increased BUN
- Group A streptococcus infection



Glomerular Disorders

Chronic Glomerulonephritis

- Gradually worsening symptoms
 - fatigue, anemia, hypertension, edema and oliguria
- Typical UA findings
 - hematuria, massive proteinuria, glucosuria, dysmorphic RBCs and presence of varieties of casts including broad cast
- Marked \downarrow GFR with \uparrow BUN and creatinine



Glomerular Disorders

Nephrotic Syndrome

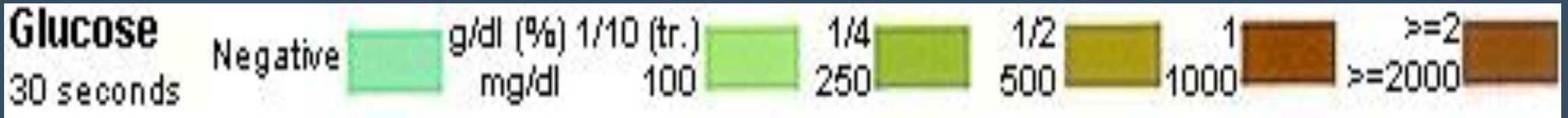
- Damage to the kidneys causes them to spill large amounts of protein into the urine
- Laboratory findings:
 - Urine dip stick: 3+ protein
 - 24 hour urine collection protein >3.5 g/day
 - Hematuria ,lipiduria, fatty casts and waxy casts, RTE casts



Nephrotic Syndrome

- Oval fat bodies, fat droplets
 - Maltese cross formation with polarized light microscopy
- Increased permeability of the glomerular membrane
 - leads to damage to the shield of negativity
 - Podocytes that produce less tightly connected barrier





- Glucose oxidase test
 - Specific test for glucose
 - Glucose oxidase \rightarrow gluconic acid and H_2O_2
 - Peroxidase \rightarrow oxidized chromogen and H_2O
- False positive: Oxidizing agents
- False negative: High ascorbic acid , high ketones, high specific gravity, low temp. and Improperly preserved specimens



Glycosuria

- Hyperglycemia-associated
 - Diabetes mellitus
 - Gestational diabetes
 - Other endocrine disorders
- Renal-associated
 - Fanconi syndrome
 - End-stage renal disease
 - Other rare causes



Fanconi Syndrome

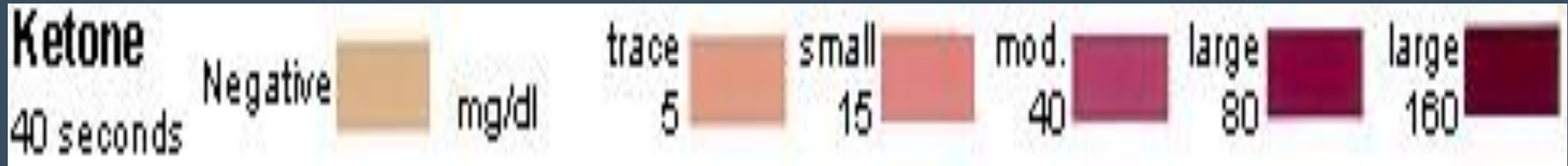
- Disorder associated with tubular dysfunction
- Failure of tubular reabsorption in the proximal convoluted tubule
- Urinalysis findings are glucose and may be mild protein in the urine
- Inherited disorder in association with cystinosis and Hartnup disease



Renal Glycosuria

- Inherited disorder
- Presence of glucose in the urine when blood glucose level reaches the maximum tubular reabsorption capacity
- Increased urine glucose with normal blood glucose





- Reaction of sodium nitroprusside with acetoacetic acid produce purple color
- False positive : levodopa and medications with free sulfhydryl groups
- False negative: Improper preservation



Acetest

- Sodium nitroprusside in tablet form
 - Confirmatory test
 - Place tablet on white paper
 - One drop of urine and wait 30 seconds for reaction
 - A purple lavender discoloration of the tablet indicates the presence of acetone Compare
 - Negative, small, moderate, large



Ketonuria

- Excretion of ketone bodies
 - β -hydroxybutyric acid, acetoacetic acid and acetone
- Increased fat catabolism
 - Inability to metabolize glucose
 - Diabetes mellitus
 - Diabetic ketoacidosis
 - Vomiting, exercise
 - Starvation





- Peroxide and chromogen (yellow) react with hemoglobin peroxidase
 - Oxidized chromogen (green)
- Free hemoglobin or myoglobin
 - Uniform pattern
- Intact RBCs
 - Speckled pattern





- False positive
 - Strong oxidizing agents
 - Bacterial peroxidases
 - Menstrual contamination
- False negative
 - Crenated RBCs (decreased RBC lysis)
 - Unmixed specimens
 - Formalin
 - High nitrite
 - High ascorbic acid
 - Certain medications



Blood in Urine

	Hematuria	Hemoglobinuria	Myoglobinuria
Urine color	Red or brown	Red or brown	Red or brown
Plasma color	Normal	Pink	Normal
Urine pad on test strip	Positive	Positive	Positive
Urine microscopic	Many intact red cells	Occasional red cell	Occasional red cell
Serum haptoglobin	Normal	Low	Normal
Causes	Glomerulonephritis, pyelonephritis, calculus, tumor, infection, sickle cell disease, trauma	Free hemoglobin(intravascular hemolysis) , transfusion reaction, hemolytic anemias, severe burns and malaria	Increased muscle breakdown, trauma, extensive exertion, muscle wasting diseases,prolonged coma, drug abuse



Tubular disorders

Acute Tubular Necrosis

- Disorder associated with damage to the renal tubules caused by ischemia or toxic agents
- Urinalysis results
 - Presence of large amount of hemoglobin and myoglobin
 - Proteinuria
 - Renal tubular epithelial cells
 - Casts may be present
 - Hyaline, granular, waxy, broad and RTE





- Diazo reaction
 - Bilirubin glucuronide reacts with diazonium salt (tan) to form azodye (pink to violet)
- False positive
 - Urine pigmentation
 - Certain medications
- False negative
 - Exposure to light
 - High ascorbic acid
 - High nitrite



Ictotest

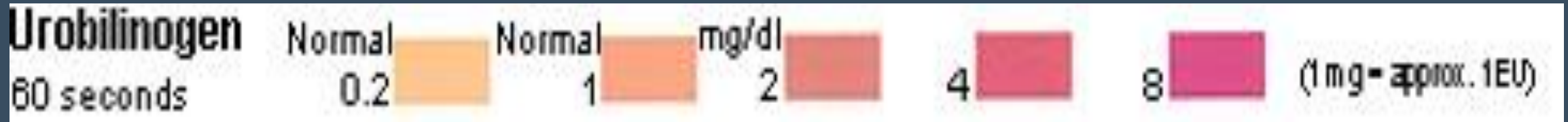
- Alternative diazo reaction in tablet form
 - More sensitive and less subject to interference
 - 10 drops of urine onto absorbent pad
 - Tablet placed on mat
 - One drop of water onto tablet (5 seconds), followed by second drop that pours onto pad
 - Observe color after 60 seconds
 - Positive - blue or purple
 - Negative - pink or red





- Multistix
 - Ehrlich's reaction
 - Urobilinogen and Ehrlich reagent react to form red compound
 - Results reported in Ehrlich units, equal to mg/dL
- Chemstrip
 - Urobilinogen and diazonium salt react to form azodye (red)
 - Results reported in mg/dL
 - More specific for urobilinogen





- Multistix
 - False positive: highly pigmented urine, ehrlich-reactive compounds (Porphobilinogen and Certain drugs)
 - False negative: old specimens and formalin
- Chemstrip
 - False positive: highly pigmented urine
 - False negative: old specimens, formalin and high nitrates



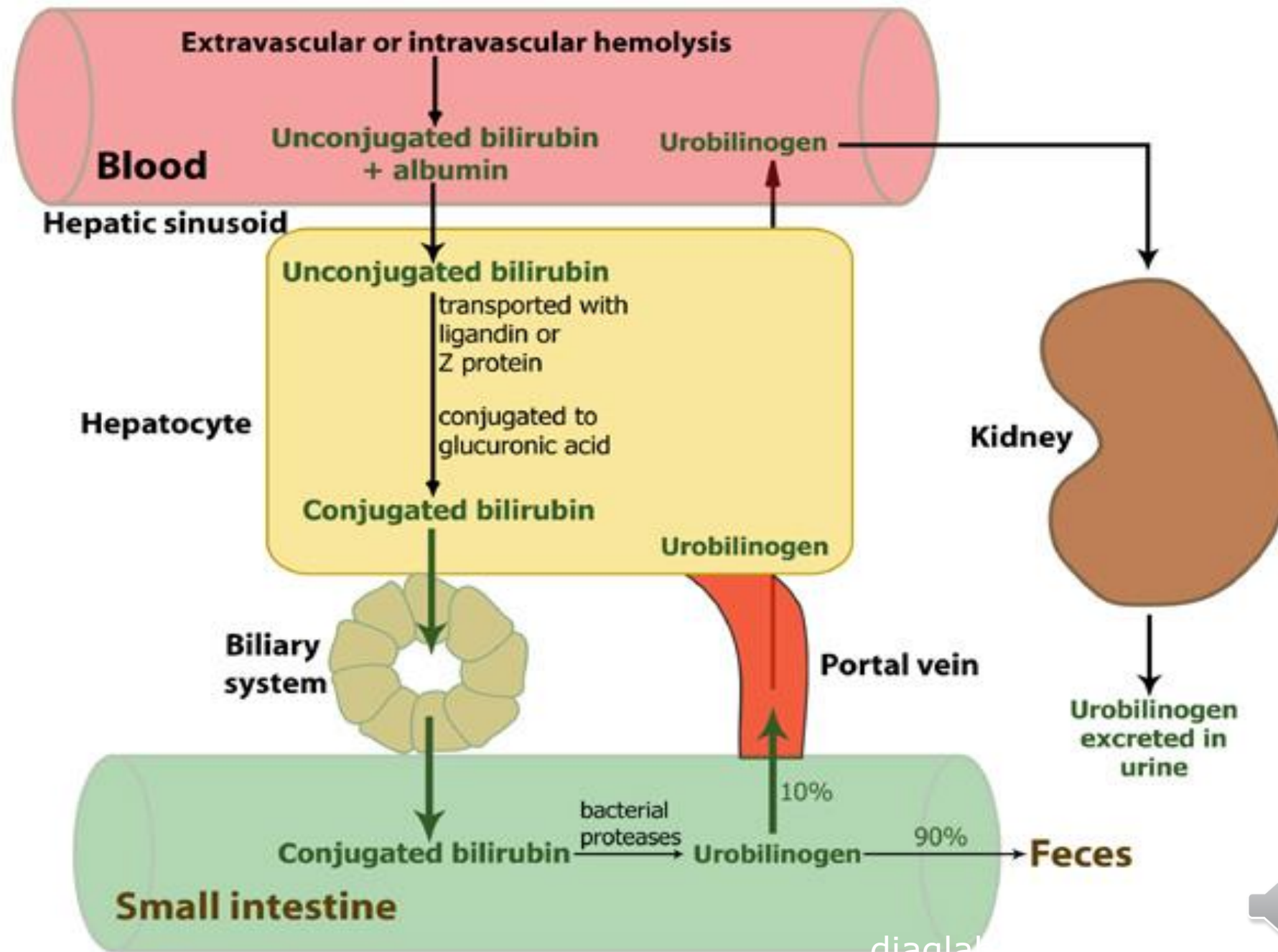
Urine Bilirubin and Urobilinogen

Detection of bilirubin in urine (along with urobilinogen) is helpful in the differential diagnosis of jaundice

	Hemolytic Jaundice	Hepatocellular Jaundice	Obstructive Jjaundice
Bilirubin	Positive	Positive /negative	Positive
Urobilinogen	Positive	Positive	Negative

- In acute viral hepatitis, bilirubin appears in urine even before jaundice is clinically present
- Presence of bilirubin indicates conjugated hyperbilirubinemia (obstructive or hepatocellular jaundice).





Nitrite

60 seconds

Negative



Positive



Positive



(Any degree of uniform pink colour is positive)

- Bacteria reduce nitrate to nitrite
- Greiss reaction
 - Nitrite reacts with aromatic amine to form diazonium salt
 - Salt reacts with tetrahydrobenzoquinolin to form azodye (pink)
- Standardized to correspond to 100,000 organisms / mL



Nitrite

80 seconds

Negative



Positive



Positive



(Any degree of uniform pink colour is positive)

- False positive
 - Old specimens
 - Highly pigmented urine
- False negative
 - Non-nitrate reducing bacteria
 - Insufficient contact time between nitrate and bacteria
 - Lack of urinary nitrate
 - Rapid reduction of nitrite to nitrogen
 - Antibiotics, high ascorbic acid or specific gravity



Lower UTI/Cystitis

- The most common symptoms associated with lower UTI include dysuria or acute pain, frequent urination, urgency, and incontinence.
- Occasionally hematuria, cloudy urine, or foul-smelling urine.
- Bacteria.



Leukocytes

2 minutes

Negative

trace

small

+

mod.

++

Large

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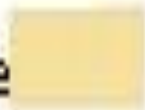
- False positive
 - Oxidizing agents
 - Formalin
 - Highly pigmented urine
 - Macrobid (antibiotic)
- False negative
 - High protein
 - High glucose
 - High oxalic or ascorbic acid
 - Other antibiotics



Leukocytes

2 minutes

Negative



trace



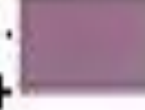
small

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mod.

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Large

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- Leukocyte esterase
 - Present in granulocytic and monocytic WBCs
 - Also histiocytes and Trichomonas
 - Esterase hydrolyses acid ester to an aromatic compound
 - Compound reacts with diazonium salt to produce azodye (purple)
 - Require 2 minutes for reaction



Acute pyelonephritis

- It's a result of ascending movement of bacteria from a lower UTI into the renal tubule and interstitium.
- Patients symptoms:
 - urinary frequency ,burning on urination and lower back pain.
- The ascending movement of bacteria from the bladder interfere with the downward flow of urine.



Acute pyelonephritis

- Incomplete emptying of the bladder during urination
- Urinalysis results:
 - numerous WBC and bacteria with mild proteinuria and hematuria
- Presence of WBC cast signifies infection within the tubules.



Acute interstitial nephritis

- It's an inflammation of the renal interstitial followed by inflammation of the renal tubules.
- Reaction to medications
- Urinalysis results:
 - hematuria, protein urea , numerous WBCs and WBC cast without bacteria.
 - Hansel stain for presence of eosinophil





- Change in dissociation constant (pK_a) of polyelectrolyte in alkaline medium
 - Release of H^+ in proportion to ions in solution
 - Higher the concentration \rightarrow more H^+ released (lower pH)
 - Bromthymol blue on pad measures pH change
 - 1.000 (alkaline) \rightarrow 1.030 (acid)





- False positive
 - High protein
- False negative
 - Highly alkaline urines
 - 0.005 added to SG value by automated readers when pH is 6.5 or higher

