CLINICO-PATHOLOGICAL EXAMINATIONS

Dr. SATISH J. CHAUDHARI
CLINICAL PATHOLOGY

A. HEMATOLOGY

B. CLINICAL CHEMISTRY
   - 1. SERUM BIO CHEMISTRY
   - 2. URINE ANALYSIS

C. CYTOLOGY

D. MYCOLOGY
HEMATOLOGY

BLOOD

FOR
(A) HEMOGLOBIN ESTIMATION
(B) PCV
(C) ESR
(D) TEC
(E) TLC

- Collection of blood
  - Vein-jugular, ear; cephalic- animals; wingvein-birds
  - Large animals- vials; Small animals- syringe, vials
  - Use of vacutainers- plain/ with anticoagulants/ gel

- Anticoagulants
## Anticoagulants

<table>
<thead>
<tr>
<th>Name</th>
<th>For 10 ml of Blood</th>
<th>For Transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA(K/Na)</td>
<td>1 ml of 1% soln (10-20 mg)</td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
<td>0.2ml of 1% soln (1-2 mg)</td>
<td></td>
</tr>
<tr>
<td>Sod. Citrate</td>
<td>1 part of 3.8% soln (10-20 mg)</td>
<td></td>
</tr>
<tr>
<td>Amm + Pol.Oxalate (</td>
<td>[1.2 gm of Am.ox. + 0.8 gm of Pot.ox.to</td>
<td></td>
</tr>
<tr>
<td>Heller’s &amp; Paul’s Double oxalate)</td>
<td>make 100 ml Water]</td>
<td></td>
</tr>
<tr>
<td>Sod. Fluoride&amp; thymol</td>
<td>100 mg of Sod. Fluoride &amp;10 mg of Thymol</td>
<td>For blood glucose (10:1)</td>
</tr>
<tr>
<td>ACD Soln (Acid citrate dextrose)</td>
<td>ACD 25 ml for 100 ml of blood (</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dextrose: 14.7 gm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trisal citrate: 13.2 gm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Citric acid: 4.4 gm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dist. Water to make 1000 ml : Autoclave)</td>
<td></td>
</tr>
</tbody>
</table>
Hb : Sahlis method / Use of spectrophotometer-Less errors

PCV : Wintrobes Method

PCV, Buffy coat, Plasma-Colour

TEC : Neubours chamber

TLC : Neubours chamber

ESR : Wintrobes tubes, Mainly in Dogs, Prognostic value

Increased ESR-Young/Old age, female, pregnancy, anemia, Inflamm.disease processes

Reticulocyte : Immature RBCs, Larger in size, Require special stain, Presence of large no.of Reticulocytes – Increased erythropoiesis. Reticulocyte Shower; - Acute Hemorrhage-Spurt in Increased erythropoiesis
**Erythrocytic Indices** :- from Hb, PCV, TEC values.

- PCV x 10
- Hb x 100
- TEC
- MCV = \( \frac{---}{TEC} \) fl
- MCHC = \( \frac{---}{PCV} \) gm%
- MCH = \( \frac{---}{TEC} \) pg

### Morphological Classification of Anaemia:

<table>
<thead>
<tr>
<th>MCV</th>
<th>MCHC – Normal</th>
<th>MCHC – Decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normocytic normochromic</td>
<td>Normocytic</td>
</tr>
<tr>
<td>Hypochromic</td>
<td>Normocytic normochoic</td>
<td>Normocytic hypochromic</td>
</tr>
<tr>
<td>Increased</td>
<td>Macrocytic normochromic</td>
<td>Macrocytic</td>
</tr>
<tr>
<td>Hypochromic</td>
<td>Macrocytic hypochromic</td>
<td>Normocytic hypochromic</td>
</tr>
<tr>
<td>Decreased</td>
<td>Microcytic normochromic</td>
<td>Microcytic</td>
</tr>
<tr>
<td>Hypochromic</td>
<td>Microcytic hypochromic</td>
<td>Normocytic hypochromic</td>
</tr>
</tbody>
</table>

- **Normocytic normochromic**: Hemolysis, Acute hemorrhage, under production
- **Macrocytic normochromic**: Maturation Arrest (B12 & Folic acid Defi.), Acute Hemorrhage/Hemolysis
- **Microcytic hypochromic**: Chronic hemorrhage, Deficiencies of Iron, Copper, Riboflavine
**Differential Diagnosis – Anaemia**

( Hb, PCV, TEC, Indices, Reticulocytes, TLC, Thrombocytes )

- **Microcytic hypochromic**
  - Chronic
  - Def. of Iron, Cu, Ribofla.
  - Hemorrhage

- **Normocytic normochromic**
  - True megaloblastic
    - Maturation arrest
      - Def. - B12, Folic acid
  - Transitory

- **Macrocytic normochromic**
  - Non-megalo
    - Increased
    - Bone marrow activity after A. hemor, hemolysis

( Reticulocyte count )

- Low
  - Anemia of under Production
  - WBC: Thromocytes
  - Low
    - Hypo/Aplastic
    - Uremia, Neoplasia
    - Irradiation
  - High/Normal
    - Infection

- High/Normal
  - Defect in RBC
    - Neoplasia
    - Infection
    - Inherited enzyme deficiency
  - No Defect in RBC
    - Metabolic, Immune
    - Reaction, Chemical
    - Hyperplastic
    - Physical agents

- Acute ( Early stage before bone marrow becomes hyperplastic

- Hemolysis
  - Blood Loss

- Hemorrhage
Errors commonly made in collection of Blood

**Haemolysis:**
- Use of Wet Syringes / Needles.
- Failure to remove needle before filling collection tube/vial.

**Lipemia:** If no adequate fasting before collection of Blood, We may find elevated values of Hb, Icterus index, TP, Transaminase.

**Precautions:**
- Blood be collected when animal is at rest & without undue excitation.
- Clotting of blood may result: If too much time is taken in obtaining blood; or failure to agitate the blood sample immediately after placing it in vial i.e. improper mixing of anticoagulant with blood. Also quantity of blood to be collected as per the concentration of anticoagulant.

When serum is desired, do not place blood in Refrigerator immediately/before Clot formation & retraction occurs; if, retards serum collection. The longer the whole blood is held before examination, greater will be the deterioration.

Serum must be separated from the clot as early as possible.
Blood Smear

Examination of a well stained smear can provide more valuable information than any other single laboratory test.

Slides must be clean, grease-free with smooth unbroken ends. Spreader slide should be used.

Blood smear should be collected at the height of Temperature, preferably resorting to any treatment and from tip of the ear / tail.

After the film is spread, hasten the drying with current of air. Rapid drying prevent crenation and fragmentation of Erythrocytes. Label the slide on opposite side of smear.

Slides must be fixed with Methanol, if examination is delayed. Wrap the slide in paper.

Leishmans Stain; Giemsa Stain

Good differential staining requires that the water must be of proper pH or adjusted to it. ( 6.8 – 7.0 )

Examin. For-

- RBCs-Size-normo, macro, microcytic : Shape-target cell, spherocyte,:
- Colour-normal, hypo, hyperchromatic :
- Abnormal-nucleated, baso, stipling, HJ bodies, polychromasia:
- Parasites-Hemoprotozoan, Filaria
- Leukocytes-granulocytes, Non-granulocytes
Leukocytes & its responses

**Leukopenia:**
- Viral infections
- Bacterial diseases – early stages
- Shock – Endotoxic, septic, anaphylactic
- Bone Marrow abnormalities – hypo-, Dysplasia, Defect. Maturation
- Chemical agents – chloromethane, streptomycin, chlorpromazine
  - Poisons - Lead, Mercury, Arsenic

**Leukocytosis:**
- Physiological: Excitement, Fear, convulsions, Digestion, Pregnancy
- Pathological: Infection – Local / generalised
  - Intoxications – uremia, Acidosis, venom
  - Tissue Necrosis - Infarction, Burn, Gangrene, neoplasm
  - Acute hemorrhage; Acute Hemolysis
  - Adrenal corticoids – Monocytosis mainly

Regenerative shift to Left; Increased TLC with increased immature forms

Degenerative shift to Left; decreased TLC with increased immature forms;
  “Unfavourable Prognosis”

Shift to Right; Increase in hypersegmented cells
## Normal Range: Haemogram

<table>
<thead>
<tr>
<th></th>
<th>Equine</th>
<th>Bovine</th>
<th>Ovine</th>
<th>Caprine</th>
<th>Porcine</th>
<th>Canine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RBCs (x10^6)</strong></td>
<td>8-13</td>
<td>5-10</td>
<td>8-16</td>
<td>12-20</td>
<td>5-8</td>
<td>5.5-8.5</td>
</tr>
<tr>
<td><strong>Hb (gm%)</strong></td>
<td>11-17</td>
<td>8-14</td>
<td>8-16</td>
<td>8-14</td>
<td>10-16</td>
<td>12-18</td>
</tr>
<tr>
<td><strong>PCV (%)</strong></td>
<td>35-58</td>
<td>24-48</td>
<td>24-50</td>
<td>24-48</td>
<td>32-50</td>
<td>37-55</td>
</tr>
<tr>
<td><strong>TLC (x10^3/cm)</strong></td>
<td>8-15</td>
<td>4-12</td>
<td>4-12</td>
<td>6-16</td>
<td>11-22</td>
<td>6-18</td>
</tr>
<tr>
<td><strong>N (%)</strong></td>
<td>45-60</td>
<td>15-45</td>
<td>10-50</td>
<td>30-48</td>
<td>28-47</td>
<td>60-77</td>
</tr>
<tr>
<td><strong>L (%)</strong></td>
<td>35-60</td>
<td>45-75</td>
<td>40-75</td>
<td>50-70</td>
<td>39-52</td>
<td>12-30</td>
</tr>
<tr>
<td><strong>M (%)</strong></td>
<td>1-8</td>
<td>2-7</td>
<td>1-6</td>
<td>1-4</td>
<td>2-10</td>
<td>3-10</td>
</tr>
<tr>
<td><strong>E (%)</strong></td>
<td>1-5</td>
<td>2-20</td>
<td>1-10</td>
<td>3-8</td>
<td>0-11</td>
<td>0-10</td>
</tr>
<tr>
<td><strong>B (%)</strong></td>
<td>0.5</td>
<td>0-2</td>
<td>0-3</td>
<td>0-2</td>
<td>0-2</td>
<td></td>
</tr>
</tbody>
</table>
## Normal Range: Serum Biochemistry

<table>
<thead>
<tr>
<th></th>
<th>Equine</th>
<th>Bovine</th>
<th>Ovine</th>
<th>Caprine</th>
<th>Porcine</th>
<th>Canine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (mg%)</td>
<td>11.2-13.8</td>
<td>8-10.5</td>
<td>11.5-13</td>
<td>9.00-11.6</td>
<td>11-11.3</td>
<td>9-12</td>
</tr>
<tr>
<td>P (mg%)</td>
<td>3.1-5.6</td>
<td>4-7</td>
<td>4-7</td>
<td>4.2-9.8</td>
<td>4-11</td>
<td>2-4</td>
</tr>
<tr>
<td>Tot.Prot (gm%)</td>
<td>6-7.7</td>
<td>5.7-8.1</td>
<td>6.0-7.9</td>
<td>5.9-7.8</td>
<td>3.5-6</td>
<td>6-8</td>
</tr>
<tr>
<td>Alb (gm%)</td>
<td>2.9-3.8</td>
<td>2.1-3.6</td>
<td>2.4-3</td>
<td>2.7-3.9</td>
<td>1.9-2.4</td>
<td>2.4 - 3.0</td>
</tr>
<tr>
<td>BUN (mg%)</td>
<td>10-24</td>
<td>10-30</td>
<td>8-20</td>
<td>10-28</td>
<td>8-24</td>
<td>10-30</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1-2</td>
<td>1-2</td>
<td>1-1.9</td>
<td>0.9-1.8</td>
<td>1-2.7</td>
<td>1-1.7</td>
</tr>
<tr>
<td>Glucose (mg%)</td>
<td>60-100</td>
<td>35-55</td>
<td>35-74</td>
<td>45-60</td>
<td>70-100</td>
<td>60-100</td>
</tr>
</tbody>
</table>
SERUM BIOCHEMISTRY ABNORMALITIES

I. Blood urea nitrogen: **Increased**

1. Prerenal disease
   - (1) Heart failure
   - (2) Shock
2. Renal disease
3. Post renal disease
   - (a) Urinary tract obstruction
   - (b) Urinary tract perforation
4. Secondary renal hyperparathyroidism
5. Pseudohyperparathyroidism (neoplasia)
6. High protein ration – nonfasting sample

**Decreased**

1. Hepatic insufficiency
2. Low protein ration
3. Malabsorption
4. Overhydration with fluids

II. Creatinine: **Increased**

In the same conditions as in BUN, except is not affected by:

- a. Protein diet or catabolism
- b. Exercise

**Decreased**

No significance
### III. Blood glucose

**Increased**
1. Diabetes mellitus
2. Pancreatitis, acute and chronic
3. Excitement, convulsions, exercise-transitory
4. Hyperadrenocorticism – mild increase
5. Corticosteroid or ACTH administration
6. Glucose infusions
7. Chronic hepatic disease
8. Hyperthyroidism
9. Hyperpituitarism
10. Recent high carbohydrate meal

**Decreased**
1. Hyperinsulinism
2. Functional hypoglycemia
4. Hypoadrenocorticism
5. Hypothyroidism
6. Hypopituitarism
7. Hepatic insufficiency – marked
8. Serum not removed from clot rapidly
9. Pregnancy toxemia
10. Malnutrition

### IV. Serum bilirubin

**Increased**
1. Hemolysis
2. Liver disease

**Decreased**
Bone marrow depression anemia
V. Total protein (TP):

**Increased**
1. Polycythemia
   a. Relative-in proportion to water lost
   b. Transient-only slight increase
2. Increased gamma globulin production

**Decreased**
1. Inadequate production
   a. Age-in young animals
   b. Malnutrition
   c. Hepatopathy
   d. Neoplasia
   e. Pregnancy and lactation
2. Loss of body protein
   a. Acute blood loss
   b. Chronic blood or protein loss
3. Loss of blood protein into body cavities

VI. Serum glutamic oxaloacetic transaminase (SGOT)

**Increased**
1. Hepatocellular disease
2. Skeletal muscle necrosis or degeneration
3. Cardiac muscle necrosis or degeneration

VII. Serum glutamic pyruvic transaminase (SGPT)

**Increased**
1. Hepatocellular disease
VIII. Calcium :–  

**Increased**

1. Primary hyperparathyroidism - rare  
2. Pseudo hyperparathyroidism  
3. Vitamin D intoxication  
4. Malignant neoplasm with osseous metastasis 

**Decreased**

1. Blood Collected in anticoagulant 
2. Secondary renal hyperparathyroidism 
3. Secondary nutritional hyperparathyroidism 

IX. Inorganic phosphorus :–

**Increased**

1. Renal insufficiency  
2. Vitamin D intoxication  
3. Hemolysis of blood sample  
4. Hyperparathyroidism  
5. Normal in young animals  
6. High-phosphorus and low-calcium ration  
7. Secondary hyperparathyroidism 

**Decreased**

1. Low phosphorus rickets  
2. Osteomalacia  
3. Primary hyperparathyroidism  
4. Low- phosphorus ration with high Ca:P ratio  
5. Hyperinsulinism
URINALYSIS

The relatively simple procedures involved in urinalysis, conducted in a few minutes of time, can yield much valuable information in regard to the function of the urinary system as well as other organs or systems of the body.

A complete urinalysis should be a routine procedure for the following:

- Surgical and geriatric patients
- Disease problems as yet without a diagnosis. Since abnormal urine findings could indicate the need for additional evaluation of a particular organ or body system

a. Kidney disease- abnormal specific gravity, proteinuria, casts, leukocytes, erythrocytes
b. End stage renal disease- low specific gravity with failure to concentrate urine when water is withheld, pH decreased
c. Bladder infection- proteinuria, leukocytes, bacteria
d. Neoplasia- exfoliated neoplastic cells, hematuria
e. Liver disease- bilirubinuria, altered urobilinogen, bilirubin crystals
f. Hemolysis- hemoglobinuria, increased urobilinogen
g. Diabetes mellitus- glycosuria and ketonuria
h. Diabetes insipidus- low specific gravity with failure to concentrate urine when water is withheld
i. Acidosis- low pH
j. Alkalosis- increased pH
Collection of urine: Clean, Sterile, Container

Timing: Early morning sample preferred
Avoid first part of urine stream
Fresh sample preferred- without preservative
If, Preservatives- Toluene, Thymol, Formalin, chloroform, Boric acid

PHYSICAL EXAMINATION

A) Volume
B) Color
C) Odour
D) Transparency

E) Specific gravity: Increased-Acute Interstitial Nephritis, Cystitis, Diabetes m, dehydration-diarrhea, fever, reduced intake, vomition
Decreased-Increased fluid intake, chronic int. nephritis, advanced stage of uremia
A. Reaction of the urine

The reaction of the urine is dependent on the diet. In vegetarians the reaction is alkaline. With carnivores and omnivores it is acidic. The normal reaction of urine among animals:

- Horse - alkaline (pH 8)
- Cattle - alkaline (pH 7.4 to 8.4)
- Sheep - alkaline
- Pig - acid or alkaline, depending upon the feed
- Dog - acid (pH 6 to 7)
- Cat - acid (pH 6 to 7)
B. PROTEIN In Urine

Normal urine does not contain protein

Proteinuria may be present in the following conditions:

a) Physiological: Is a transient episode
   (i) Excessive muscular exertion
   (ii) Excessive ingestion of proteins
   (iii) In time of emotional stress
   (iv) In convulsion

b) Pathological: In nephritis, because of increased permeability of the glomerular filter:
   Acute interstitial nephritis - there is protein in the urine with the casts
   Chronic interstitial nephritis - slight proteinuria, with casts
   Pyelonephritis; marked proteinuria with leucocytes and red blood cells
   Nephrosis: due to poisoning by phenol, arsenic, lead, phosph., Mercury sulphonamide, turpentine, etc.

   In renal congestion due to: cardiac congestive failure, ascites, tumors,

   In amyloidosis, renal infarction and neoplasms also there is proteinuria

Post renal conditions in which proteinuria is present:
   Cystitis; pyelitis; vaginal and preputial discharge;
   Urethritis; Ureteritis; Urolithiasis; Trauma and hemorrhage
C. GLUCOSE

Normally urine does not contain any glucose. All of it that is found in the glomerular filtrate is reabsorbed by the tubular epithelium since glucose is a threshold substance. Glucose in urine is called glycosuria. It is decided by Benedict’s test.

Glycosuria is present in the following conditions:

1) In emotional states - fear, excitement,- there is sudden release of adrenaline which causes hyperglycemia with resultant glycosuria.
2) Heavy meal of carbohydrates
3) After general anesthesia
4) Diabetes mellitus
5) Hyperthyroidism- due to rapid absorption of glucose from the bowel
6) Chronic pancreatitis
7) Acute pancreatic necrosis
8) Hyperpituitarism- produces hyperglycemia
9) Overactivity of adrenal cortex
10) Shock
11) Administration of glucose intravenously
12) Chronic liver disease
13) Enterotoxemia in sheep

False positive reaction for glucose can be given if other reducing agents are present: antibiotics, lactose, ascorbic acid, salicylates, chloral hydras, morphine, formaldehyde, uric acid.
D. Ketone bodies in urine

These are Acetoacetic acid (CH3COCH2COOH)
Beta hydroxyl butyric acid (CH3CHOHCH2COOH)
Acetone (CH3COCH3)

These are not normally present in the urine. But when fat metabolism is impaired (due to deficient carbohydrates) ketone bodies accumulate in the body giving rise to ketonemia and then ketonuria results.

Ketones in urine are detected by Rothera’s test.

Ketonuria is present in:
1. Diabetes mellitus - in dog and cats
2. In starvation
3. High fever
4. In cows it is met with in acetonemia, milk fever and in anorexia of high yielding animals
5. In ewes in pregnancy disease
6. Cachectic conditions
E. Blood

Hematuria: presence of whole blood in urine. Hemoglobinuria: presence of hemoglobin in urine. These can be differentiated by centrifuging a specimen of urine. In hematuria, the red blood cells will be layered at the bottom while the supernatant liquid is colorless. In hemoglobinuria in which the erythrocytes are laked, the whole fluid will be red.

**Hematuria is encountered in the following conditions**

- Pyelonephritis
- Acute nephritis, Ureteritis, Cystitis, Pyelitis
- Urolithiasis, Passive congestion of kidney
- Infection of kidney, Abscess of kidney
- Neoplasms of kidney, bladder or prostate
- During estrus. At post partum in females
- Prostatitis
- Trauma to urethra- during improper catheterisation
- Severe infections as in anthrax, leptospirosis, Rubarth’s disease
- Toxic chemical agents: copper poisoning, phenol, sulphonamide, mercury, poisoning, arsenic poisoning, thallium poisoning.
- Sweet clover disease
- Shock- capillary hemorrhage
- Parasites- Dioctophyma renal in dogs

If blood is found in the last drops of urine then the source is bladder
If the urine is red throughout, the source of blood is kidney
If the first portion of the urine is red, then the source of blood is some urethral lesion
Hemoglobinuria:
In this condition, there is increased hemolysis and is found in the following conditions
1. Leptospirosis
2. Post parturient hemoglobinuria
3. Babesiosis
4. Photosensitisation
5. Bacillary hemoglobinuria *Clostridium hemolyticum*
6. Chemical agents: Copper, mercury, sulphonamides
7. Severe burns
8. Hemolytic disease of the new born
9. Incompatible blood transfusion
10. Drinking large volume of water in cattle
11. Plant poison: Ranunculus, ash, hazel, hellebore etc

In myoglobinuria (azoturia) when the urine contains myoglobin, a positive test is obtained with the benzidine test. This can be differentiated from hemoglobinuria by the absence of erythrocytes in the sediment.

**Microscopic examination**

Epithelial cells, Blood cells, Casts, mucous threads, Bacteria, Fungi
Cytology: Effusions-pleural, pericardial, peritoneal

Exposed lesions - Scraping, Touch impressions

Aspiration Biopsy

Format for sending Samples to Laboratory:-

1. Name & address of the owner
2. Animal: Species, Breed, Age, Sex, Colour, Id. No./mark
3. Clinical History/Findings (specify duration of illness)
4. Treatment given (specify about response to treatment)
5. Samples collected (specify preservative added)
6. Disease suspected
7. Tests to be carried out