STUDY OF NORMAL CONSTITUENTS OF URINE

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Physical Characteristics

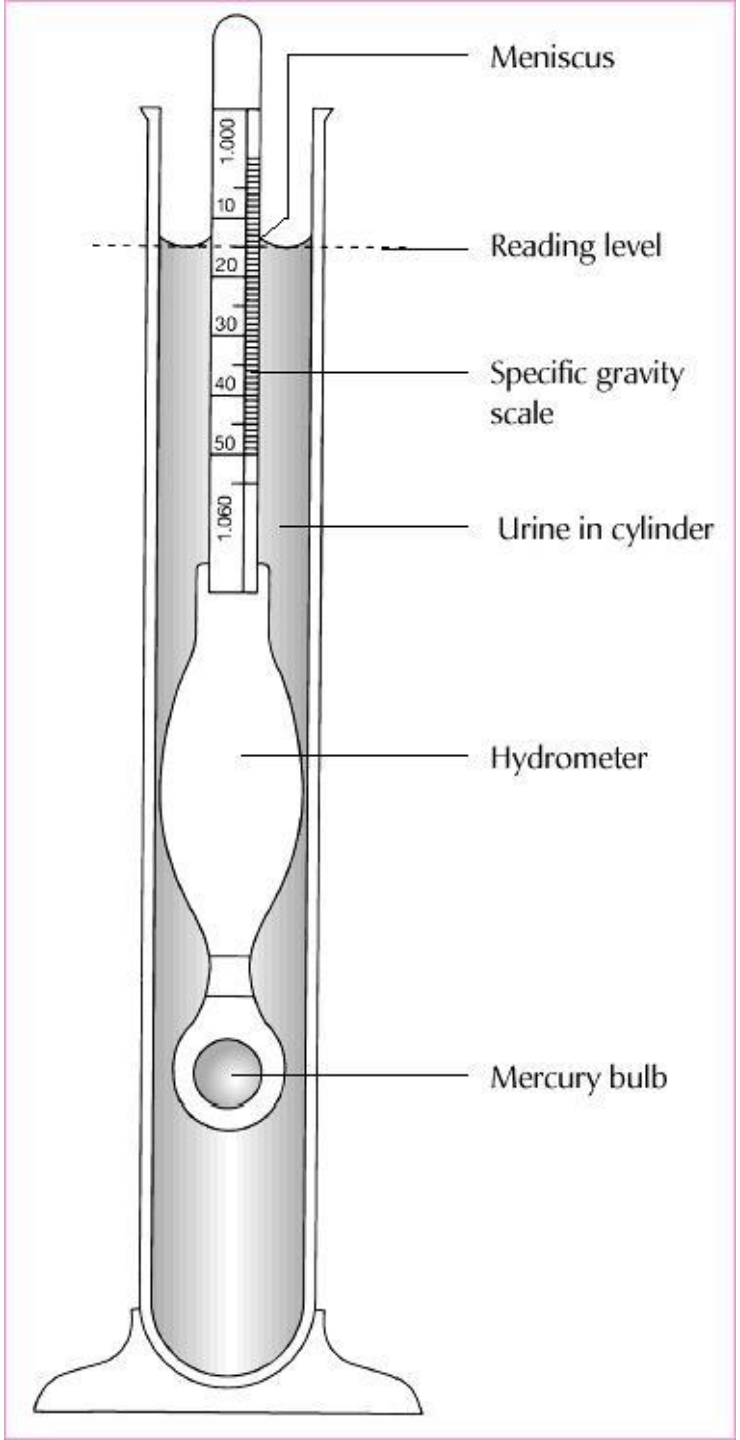
Features	Characteristic	Reason
Volume	800-2500 ml/day Average 1500 ml /day	Quantity of Fluid & Food intake, Climate Change, Physical Exercise
Appearance	Fresh Sample - Clear & Transparent	On standing, Urine appears Turbid. Bacterial Action converts urine Urea to Ammonia. This makes urine Alkaline leading to precipitation of Phosphate, Oxalates and Urates.
Color	Fresh Sample – Straw / Amber color	Presence of Pigment Urochrom imparts color to the urine.
Odor	Aromatic Odor	Presence of Volatile Organic Acids. On standing, urine gives ammonical smell due to conversion of urine Urea in Ammonia by Bacterial Action.
pH	Range : (4.8 – 7.5) Average 6.0 (Acidic)	Protein Diet makes more Acidic Urine. Diet Rich in fruits and vegetables makes Alkaline Urine. On standing Urine becomes Alkaline due to Ammonia Formation.
Specific Gravity	1.012 – 1.024	Fluid Intake and Specific Gravity has Reciprocal Relationship.

Determination of Specific gravity by urinometer

- 1. Check the urinometer by taking the Specific gravity of given sample of urine. See that urinometer does not touch to any side of or bottom of the urine jar.
- 2. Correction of Specific gravity.
- Note the room temperature (Generally urinometer is calibrated at 20 ^{o}C)
- Find out the difference between the room temperature and calibrated temperature.

- Now, Calibrated specific gravity at 20 ^{o}C = 1.010
- i. For every 3 ^{o}C rise in temperature add 0.001 as a correction factor from observed specific gravity.
- ii. For every 3 ^{o}C fall in temperature subtract 0.001 as a correction factor from observed specific gravity.

- Calibrated temperature= 20 ^{o}C for every 3 ^{o}C rise in temperature, correction factor =0.001
- Room temperature=32 ^{o}C for 12 ^{o}C correction factor = 0.001 x 4 = 0.004
- Rise temperature=12 ^{o}C
- Corrected or true specific gravity at 32 ^{o}C = Observed specific gravity + Correction Factor = **1.010 + 0.004 = 1.014**


Meniscus
1.000
10
Reading level
20
30
Specific gravity scale
40
50
1.060
Urine in cylinder
Hydrometer
Mercury bulb

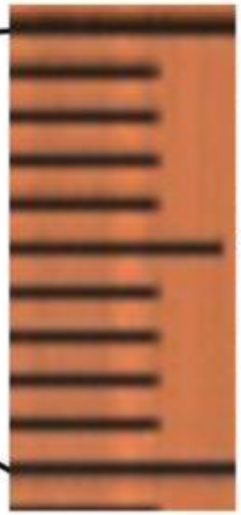
URINOMETER

Parts

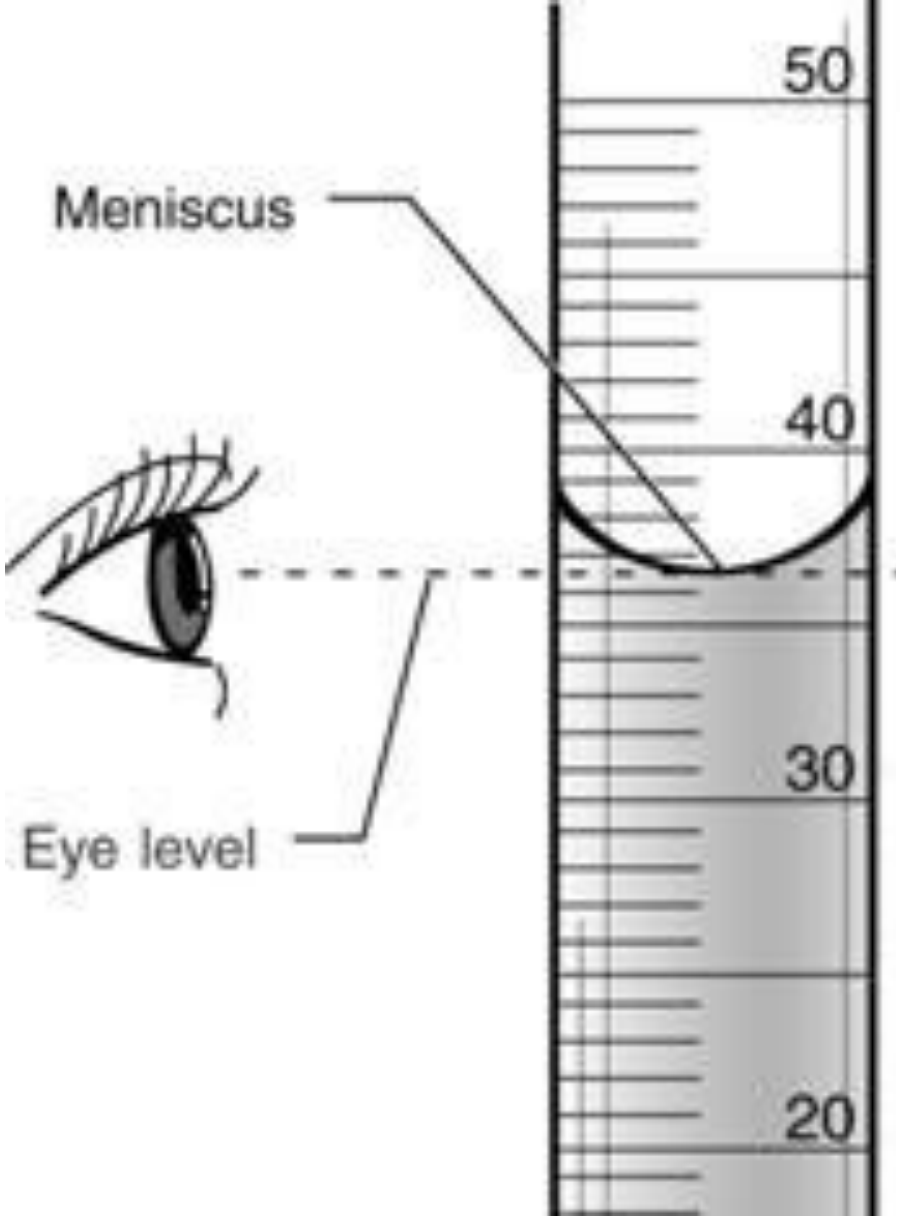
STEM

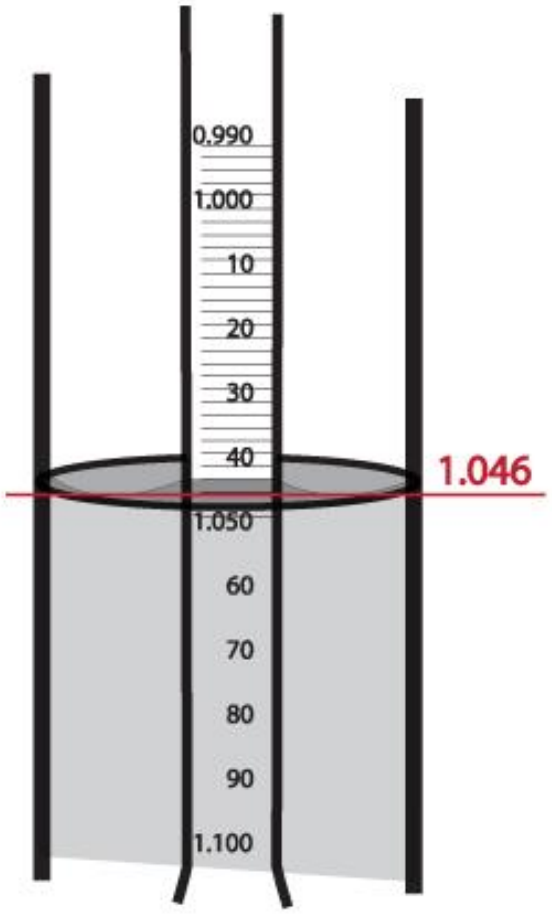
FLOAT

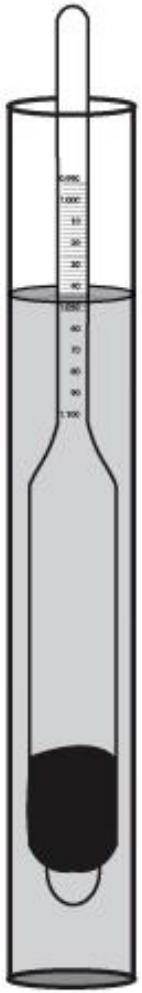
WEIGHT



@VijayPatho


Meniscus
50
40
30
20
Eye level


0.990
1.000
10
20
30
40
1.046
1.050
60
70
80
90
1.100



7. Sedimentation

- Normal urine does not contain any sediment but upon standing, sediments appear. This is due to bacterial fermentation of urea to ammonia and carbon dioxide by the action of specific enzyme urease.

Chemical composition

- Normal urine contains both organic & inorganic constituents.
- **Inorganic constituent includes** Na^{+}, Cl^{-}, K^{+}, Ca^{++}, Mg, SO_4^{-}, NH_3 & traces of bicarbonate ions.
- **Organic constituent includes** urea, uric acid, creatinine, urobilinogen, hippuric acid, indican, ethereal sulphate etc.

TESTS OF ORGANIC CONSTITUENTS

A. TEST FOR UREA

1. **Sodium Hypobromide Test:**

- When urea is treated with sodium hypobromite, it decomposes to give nitrogen.

1. Sodium Hypobromide Test

Test	Observation	Inference
To 2 ml of urea solution in a test tube, add 5 drops of 40% sodium hydroxide + 8-10 drops of bromine water.	Brisk effervescence of Nitrogen gas is observed	Urea is Confirmed. Normal Urine Contains Urea.

2. Specific Urease Test

- When urea is treated with the enzyme urease, it is converted to ammonia and corbonic acid. Under the pH of the reaction conditions, they are converted to ammonium carbonate.
- The pH of the solution goes above 8.5 when urea is converted to ammonium carbonate. At this pH, phenolphthalein gives pink colour.

2. Specific Urease Test

Test	Observation	Inference
To 5 ml urea solution in a test tube, add 2 ml of Urease suspension. add 2 drops of Phenolphthalein indicator and Observe	Pink colour is obtained	Urea is Confirmed. Normal Urine Contains Urea.

B. Test for uric acid

- Uric acid is a reducing agent in alkaline condition. it reduces phosphotungstic acid to tungsten blue.

3. Benedict's Uric Acid Reagent Test/ Phosphotungstic acid reduction test

Test	Observation	Inference
To 2 ml of uric acid solution, add 5 drops of Benedict's uric acid reagent, followed by 5 drops of 20% Na2CO3.	Blue colour is obtained	Uric acid is confirmed. Normal urine contains uric acid.

C. Test for Creatinine

- **4. Jaffe's Test:** Creatinine present in urine reacts with picric acid in alkaline medium to form creatinine picrate complex which has orange red colour.

4. Jaffe's Test

Test	Observation	Inference
To 2ml Creatinine solution + 2 ml saturated picric acid solution + 1 ml of 10% NaOH.	Orange colour is obtained	Creatinine is Confirmed. Normal Urine Contains Creatinine.

INORGANIC CONSTITUENTS

D. Test for chloride

- **Reagent Required**: **-** concentrated HNO_3 & 5% $AgNo_3$ solution.
- **Reaction**: **-**Chloride present in urine react with silver nitrate to form white ppt of silver chloride.
- $NaCl + AgNO_3 \longrightarrow AgCl + NaNO_3$

5. Chloride Test

Test	Observation	Inference
1 ml Urine sample few drops of conc. HNO_3 + 1ml $AgNO_3$. Mix it well and observe.	White curdy precipitate is obtained.	Chlorides are precipitated as AgCl. Therefore normal urine contains chloride.

E. Test for Sulphate

- **Reagent Required: -** $BaCl_2$ & concentrated HNO_3.
- **Reaction**: **-** Sulphate present in urine reacts with barium chloride solution to form white precipitate of barium sulphate.

6. Sulphate Test

Test	Observation	Inference
3 ml Urine sample + 5 drops of 10% acetic acid + 2-3 ml of Barium Chloride (2%). Mix it well and observe.	White precipitate is obtained.	Inorganic sulphates are precipitated as $BaSO_4$. Therefore normal urine contains sulphate.

F. Test for Phosphate

- **Reagent Required :-** Conc. HNO_3, NH_4OH, Ammonium molybdate solution,
- **Reaction:-** Phosphate present in urine when heated in presence of conc. HNO_3 reacts with ammonium molybdate to form canary yellow coloured precipitate of phospho-ammonium molybdate.

7. Phosphate Test

Test	Observation	Inference
3 ml Urine sample + 5 drops conc. HNO3. Add a pinch of ammonium molybdat, warm.	Canary yellow coloured precipitate of ammonium phosphomolybdate is obtained.	Phosphate is confirmed. Therefore normal urine contains phosphate.

G. Test for Calcium

- **Reagent Required**:- Conc. HNO_3, NH_4OH, Ammonium oxalate.
- **Reaction**:- Calcium present in urine reacts with ammonium oxalate in presence of conc. HNO_3 to form white precipitate of calcium oxalate.

8. Calcium Test

Test	Observation	Inference
3ml Urine + 5 drops of 10% acetic acid + 3 ml of potassium oxalate	Trace amount of White precipitate of calcium oxalate is obtained.	Calcium is present. Therefore normal urine contains calcium.

9. Ammonia Test

Test	Observation	Inference
To 5 ml of the urine sample, add 2 ml of 5% NaOH. Boil. Hold a Filter paper dipped in phenolphthalein at near the mouth of tube.	Filter papre turns pink due to liberation of NH3.	Ammonia is present. Therefore normal urine contains Ammonia.

CONCLUSION

- Normal urine is pale yellow or straw in colour
- Normal urine is acidic in nature. Normal urine has aromatic odour.
- Normal urine contains organic constituents like urea, uric acid and creatinine.
- Normal urine contains inorganic constituents like chloride, sulphate, phosphate and calcium.