Troubleshoot and Errors During Urinalysis In Clinical Biochemistry Laboratory

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Pre-Analytic Variation

Analytic Variation

Post-Analytic Variation

Pre-Analytic - Urinary Sample Collection

- **Early Morning - Fasting - Clean**
  - Microscopic Examination & Abnormal Constitutes - Protein
- **2 hours / 12 hours / 24 hours specimen**
- **24 hour specimen** = 3 -4 litre = Preferable because of very high biological variation.
- **Double Voided Sample** - Specially for Glucose Estimation - GTT
- **Midstream** - Bacteria
- **Cathere sample - can not be use for chemical constitutes**
- **Suprapubic sample** - should be after proper decontamination
- **Fecal - contamination**

Collection in infant - Scrotal & Perineal Area

After Cleaning Scrotal & Perineal Area - Apply Over It

Diet & Water Intake - Does Variation

- Especially in Spot Urine Collection
- According to Specific Analyte
- 5-HIAA
  - Bananas , Walnuts, Pineapples , Acetaminophene ,Cough Syrup - Guaifenesin
- Specific Gravity
  - Water Intake

Container & Preservative

- **Sterile Plastic Container With Preservative**

  Boric acid / Hydrochloric acid / Sulphuring acid / Formalin

- **Mixture Tablet** - — Can not be used for Electrolyte analysis

Principle To Acidify Urine
10 ml HCL - 6 mol/l per 24 hours urine excretion
It may formation of URATE
Not Suitable for Uric acid

- **NaOH & $NaHCO_3$**

  - Porphyria , Urobillinogen and Uric acid - pH 8-9

- **Most Successful with refrigeration**

Analytic Variability

Broad Range of Analyte

- Sodium : 40-220 mmol/day (20 - 180 mmol/L)
- Potassium : 25 - 125 mmol/L
- Urea : 300 - 3000 mg/dl (12 - 20 gm /day)
- Creatinine : 25 - 225 mg /dl (500 - 2000 mg / day)

Analyser should ——————

- **Lower detection Limit to Detect Lower Range**
- **Good linearity to Detect Higher Range**
- So There is no specific analyser

Dip Stick Test - Manual / Analyser

- 10 parameter - Fresh sample
- **Protein** (Tetrabromphenol blue + Citrate - pH 3 buffer) (Yellow to Green)
  - **Lower detection limit upto 150 - 300 mg /L**
  - **85% specificity**
- **Albumin** - (Tetrabromosulfonephthalein - pH 1.5)

- **Less Sensitive**
  - Globulin
  - Bence Jones Protein
  - Mucoproteins

Dip Stick Test - Manual / Analyser

Haemoglobin - (Tetramethyl Benzidine + Peroxide)

- Water can give false positive test

Glucose - GOD-POD with Potassium Iodide

- Range : 72 to 2018 mg / dl

Leukocyte : Pyrrole amino acid ester with Diazonium Salt

- False negative
  - ✓ High Glucose , Specific Gravity ,Oxalo acid
  - ✓ Cephalosporine drugs,
  - ✓ Tetracycline

Nitrate - Bacterial growth -

- False Negative in
  - ✓ high Specific Gravity & High Ascorbic acid

Dip Stick Test - Manual / Analyser

Ketone Body

- Insensitive to Beta-Hydroxybutyrate & Acetone
- 5 to 160 mg/dl —- Detection limit

pH - (Bromthymol Blue + Methyl Red)

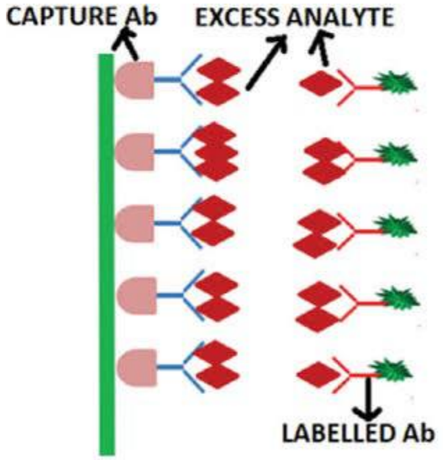
- Methyl Red 4.2 - Red >>>>>>>> 6.2 - Yellow
- Thymol Blue 6.0 - Yellow >>>>>>>> 7.6 - Blue
- In Between , Orange (lower) & Green (higher)

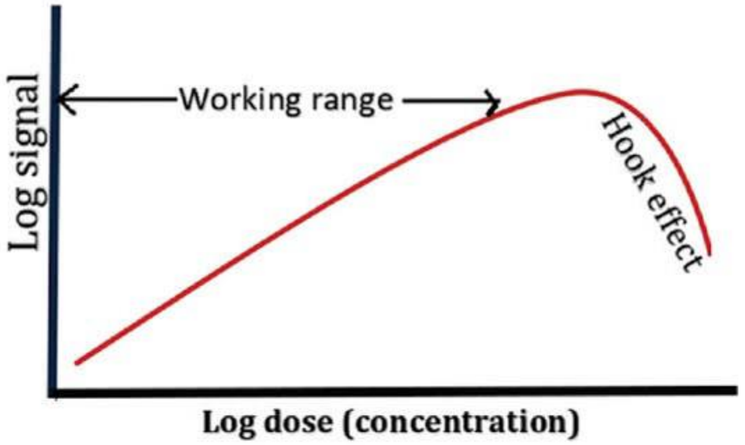
Urinary Protein - Fully Automated Chemistry Analyser

Method of Analysis

- ✓ Lowry method
- ✓ Sulfosalicyclic acid
- ✓ Turbidometry
- ✓ Benzethonium chloride
- ✓ Coomassie Brilliant Blue
- ✓ Pyrogallol red

- **Turbidometry Method**
  - High Protein - Antigen Excess effect
- **Dye Binding Method**
  - Not Specific and Not sensitive





Urinary Protein - Fully Automated Chemistry Analyser

- **Pyrogallol = Detection Limit 5 mg/dl to 600 mg/dl**
- **Biuret = Detection Limit 0.5 gm/dl to 16 gm/dl**

How do we modify approach towards testing ?

Urinary Protein - Fully Automated Chemistry Analyser

- **Pyrogallol = Detection Limit 5 mg/dl to 600 mg/dl**
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How do we modify approach towards testing ?

1. Find upper detection limit of your method
2. Run sample with High Sensitive method.
3. If Result is above upper detection limit , than Re-run sample with method of higher detection limit.

Urinary Protein

Intra Individual Variation : 30% - 50%

Diurnal Variation 50% - 100%

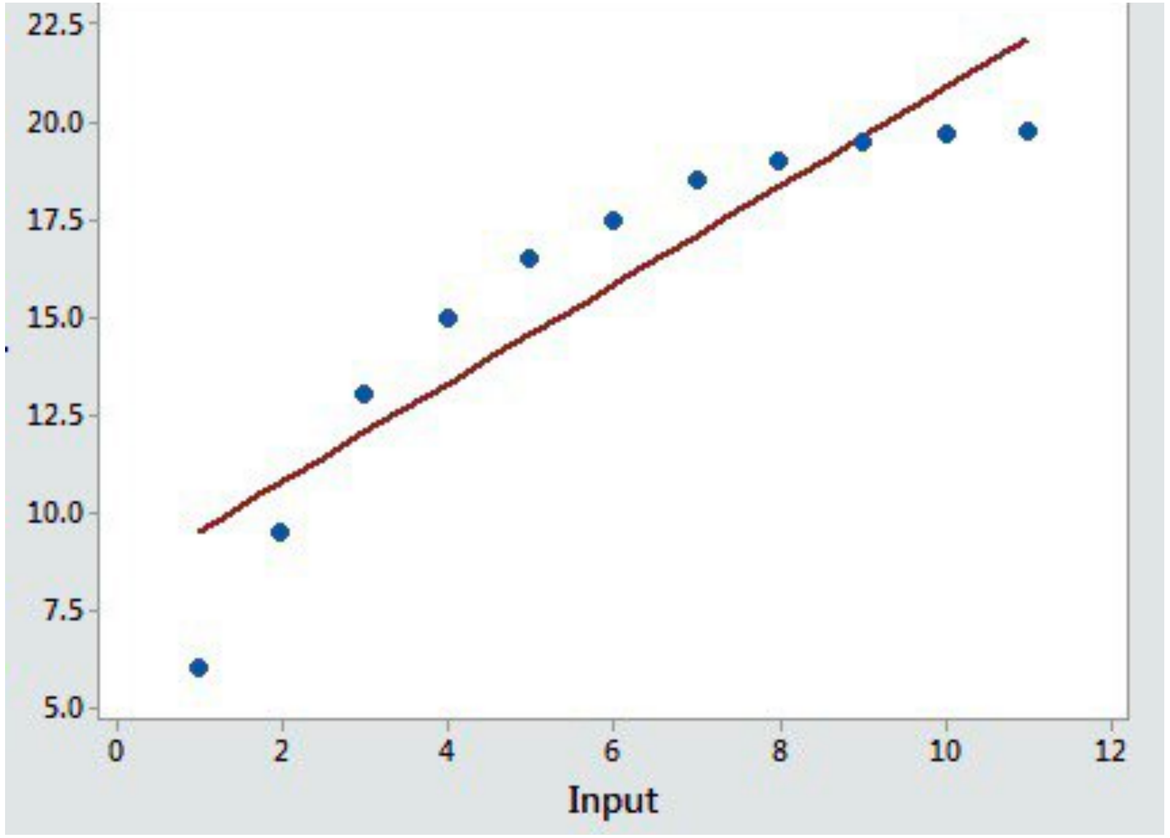
Three separate specimen should be collected on different days.

False Increase Protenuria

- After Exertion
- Urinary Tract Infection
- Acute Infection
- Immediate after Surgery
- Acute Fluid Over Fluid

Trouble In Calibration

- **At Lower Detection Limit**
- **At High Linearity Limit**
  - Spiking with Same Matrix and Find Upper Limit
- Same Matrix
- Same Interference



Quality Control for Urine Analyse

Single Use POCT - Card / Stick Test

- ✓Not Possible
- ✓Lot Verification
- ✓After Major Maintainance - Calibration - Break Down
- ✓Verify Lot Only - Manufacturer has to give ‘’Performance Data’’

Urine analysis Through Analyser - Fully - Semi Auto Analyser

- ✓As per NABL-112
- ✓2 Level at pick hours and than 1 level every 8 hourly (24 hours working Lab)
- ✓2 Level once in day (Less than 24 hours working Lab)

Total Allowable Error - Urine vs Blood

	Urine	Blood / Serum
Albumin	46%	10%
Ammonia	29.6	10%
Calcium	34%	10%
Creatinine	28%	15%
Nitrogen	18.4%	
Phosphate	22%	10%
Potassium	28%	6% / 0.5 mmol
Protein	40%	10%
Sodium	32%	5% / 4 mmol/l
Specific Gravity	0.6%	
Uric acid	24%	12%
Urea	22%	10%

Interpretation

Ketone Body

In Serum, With DKA

- Beta-Hydroxybutyrate & Acetoacetate = Ratio 6 : 1

Rothera Test : Acetone & Acetoacetate

- Semi Quantitive >>>>>>>> Quantitive ????? ——————————————>>>>
- Nitroprusside is more sensitive to Acetoacetate

Kit Measure - Enzymatic Method

- Beta-Hydroxybutyrate & Acetoacetate

QC Control With Value

Urine QC from Pool and Spike of Sodium and Potassium

Ratio

- **Protein / Creatinine Ratio**
  - 15 mg/mmol
  - < 0.2
- **Albumin / Creatinine Ratio**
  - < 30 g/mg

GFR categories (mL/min/1.73 m²), description and range				Persistent albuminuria categories, description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30–300 mg/g 3–30 mg/mmol	>300 mg/g >30 mg/mmol
	G1	Normal or high	≥90	*Low risk*	*Moderately increased risk*	*High risk*
	G2	Mildly decreased	60–89	*Low risk*	*Moderately increased risk*	*High risk*
	G3a	Mildly to moderately decreased	45–59	*Moderately increased risk*	*High risk*	*Very high risk*
	G3b	Moderately to severely decreased	30–44	*High risk*	*Very high risk*	*Very high risk*
	G4	Severely decreased	15–29	*Very high risk*	*Very high risk*	*Very high risk*
	G5	Kidney failure	<15	*Very high risk*	*Very high risk*	*Very high risk*

Urinary Osmolality

Formula : (2*Na) + (2*K) + (Urea*0.167) + (Glucose/18)

Reference Range : 500 - 800 mOsmo/kg

thank you