Sodium Test
Procedure No. FT728

Quantitative, Colorimetric Determination of Sodium in Serum, Plasma or Urine

Summary and Principle

Prior to flame photometry and ion-selective electrodes, the most popular method of determining sodium in body fluids involved its precipitation as the triple salt, sodium uranyl zinc acetate. This technique was introduced by Kolthoff[1] in 1927, with subsequent utilization of the precipitate in several ways. One approach was the colorimetric measurement of the solubilized residue itself, either directly, as reported by Albanese and Lein,[2] or by monitoring the color fade of the yellow supernate after precipitation, as described by Bradbury.[3]

The method presented is essentially an adaptation of the latter scheme,[3] wherein sodium is precipitated from a protein-free supernate as the triple salt. The resulting decrease in absorbance of the supernate-color reagent mixture is proportional to sodium content of the specimen.

Reagents

Sodium Color Reagent, Cat. No. **FT728a**
Solution of uranyl acetate, 5.3 g/dL, and zinc acetate, 15.4 g/dL, in aqueous acetic acid-ethanol mixture.
Precipitating Reagent, Cat. No. **FT728b**
Aqueous solution of trichloroacetic acid (TCA), 10 g/dL.
Sodium Standard - (140 mmol/L), Cat. No. **FT728s**
Sodium chloride, 4.091 g/L, in aqueous TCA. Equivalent to sodium value of 140 mmol/L when used as directed in method presented.

Precautions: For In Vitro Diagnostic Use.
Use care in handling Precipitating Reagent and Sodium Standard since they are mildly caustic.

Reagent Storage and Stability: All reagents are stable at 15-25°C until expiration date on labels.

Materials Required But Not Provided

Spectrophotometer capable of absorbance readings at 420 nm
Centrifuge with high speed capacity (>1500 rpm) Pipets capable of accurately delivering 0.5 and 2.5 mL
Test tubes & Cuvets Vortex mixer (optional)
Interval timer

Specimen Collection and Preparation

Serum: Remove from clot promptly and carefully to prevent hemolysis.
Plasma: Use lithium heparinate, ammonium heparinate, or lithium oxalate as anticoagulant.
Urine: Dilute portion of a well-mixed and measured 24-hour collection 1:10 (1+9) with distilled water. Depending on sodium content, a dilution of 1:5 (1+4) or 1:2 (1+1) may be required.

Sample Stability: Sodium levels remain stable for at least 14 days at 15-25°C.[4]

Interfering Substances: Contaminated glassware is the greatest source of error. All glassware should be washed with 10-20% nitric acid, rinsed thoroughly with distilled water, dried and stored in dust-free area.

Procedure

Preparation of Protein-Free Supernate
1. To properly labeled test tubes, add 0.5 mL serum plasma or diluted urine. (**Do not use Standard in this step!**)
2. Add 0.5 mL Precipitating Reagent dropwise to each tube with vigorous mixing (vortexing suggested).
3. Allow to stand for 5 minutes, then centrifuge at high speed for 5-10 minutes.

Test Procedure

1. Pipet into marked tubes the following volumes (mL), mixing promptly after each addition of the color reagent:

	REAGENT BLANK (RB)	STANDARD (S)	SAMPLE (U)
Distilled Water	0.5		
Standard		0.5	
Supernate			0.5
Color Reagent	2.5	2.5	2.5

2. Again re-mix contents of all tubes.
3. Incubate tubes for 10 minutes at room temperature (15-30°C).
4. After incubation period, mix thoroughly and centrifuge at high speed for 5 minutes.
5. Carefully transfer supernate of each tube to appropriate cuvet.
6. With spectrophotometer set at 420 nm, **zero the instrument with water.** Read and record the absorbance of the Reagent Blank (RB), Standard (S) and Unknowns (U) within 30 minutes.

Quality Control: Control sera and/or urines, assayed for sodium content by this method, flame photometry or ion-selective electrode methods, should be included with each set of unknowns. Stanbio Ser-T-Fy I, Normal Control, Cat. No. G427-86 and Stanbio Ser-T-Fy II, Abnormal Control, Cat. No. G428-86 are recommended for each test run.

Results

Values are derived by the following calculation:

$$\text{Serum, Plasma or Urine Sodium (mmol/L)} = \frac{\text{Abs (RB)} - \text{Abs (U)}}{\text{Abs (RB)} - \text{Abs (S)}} \times 140$$

Where Abs (RB), Abs (U) and Abs (S) represent the absorbance of the Reagent Blank, Unknowns, and Standard, respectively, and 140 the equivalent value of the sodium standard in mmol/L.
Example: A serum sample assayed by the method described gave an absorbance reading of 0.936, with the Reagent Blank reading 1.385 and the standard reading 0.967. Therefore:

$$\text{Sodium (mmol/L)} = \frac{1.385 - 0.936}{1.385 - 0.967} \times 140 = 150$$

NOTE: Urine values must be multiplied by the appropriate dilution factor.

$$\text{Urine Sodium (mmol/24h)} = \frac{\text{Urine Sodium (mmol/L)} \times \text{24h volume (mL)}}{1000}$$

Precision: Multiple assays (n = 20) on a serum pool (mean = 100.7 mmol/L) over an 11 day period revealed a standard deviation (SD) of 1.5 mmol/L and a coefficient of variation (CV) of 1.4%.

Correlation: Each of six serum pools were assayed for sodium by flame photometry (range: 126-149 mmol/L). Replicate analyses (n = 6) using the method presented revealed an average deviation from the reference method of - 1.5 mmol/L.

Linearity: When performed as directed, the method is linear from 0 - 160 mmol/L.

References

1. Kolthoff IM: Z Anal Chem 70:397, 1927
2. Albanese AA, Lein MD: J Lab Clin Med 33:246, 1948
3. Bradbury JT: J Lab Clin Med 31:1257, 1946
4. Weissman N, Pilegg VJ: IN Clinical Chemistry - Principle and Technics, 2nd ed. RJ Henry et al. Eds. Harper & Row, New York, 1974, p642-643
5. Interchim Laboratory data

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