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SODIUM REAGENT SET (COLORIMETRIC METHOD)

INTENDED USE
For the colorimetric determination of sodium in human serum and plasma.

INTRODUCTION
Sodium is the major cation of extracellular fluid. It plays a central role in the maintenance of the normal distribution of water and the osmotic pressure in the various fluid compartments. The main source of body sodium is sodium chloride contained in ingested foods. Only about one-third of the total body's sodium is contained in the skeleton since most of it is contained in the extracellular body fluids.[1, 2]

Hyponatremia (low serum sodium level) is found in a variety of conditions including the following: severe polyuria, metabolic acidosis, Addison's disease, diarrhea, and renal tubular disease. Hypernatremia (increased serum sodium level) is found in the following conditions: hyperadrenalism, severe dehydration, diabetic coma after therapy with insulin, excess treatment with sodium salts.[1, 2]

PRINCIPLE
The present method is based on modifications of those first described by Maruna[3] and Trinder[4] in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

REAGENT COMPOSITION
1. Filtrate Reagent: Uranyl Acetate 2.1 mM and Magnesium Acetate 20 mM in ethyl alcohol.
2. Acid Reagent: A diluted acetic acid.
3. Sodium Color Reagent: Potassium Ferrocyanide, non-reactive stabilizers, and fillers.
4. Sodium Standard: Sodium Chloride solution: 150 mEq/L of sodium

REAGENT DETERIORATION
If turbidity has occurred, turbidity may be a sign of contamination.

WARNING AND PRECAUTIONS
1. The reagent is for *in vitro* diagnostic use. **Caution**: Do not pipette the solution by mouth. Avoid ingestion/contact.
2. Specimens should be considered infectious and handled appropriately.

STORAGE AND STABILITY
Store all reagent set at room temperature (15 - 30°C). The reagents are stable until the expiration date indicated on the label.

SPECIMEN COLLECTION
Freshly drawn serum is the specimen of choice and a 50 µl (0.05 ml) amount is required. Plasma from non-sodium containing anticoagulants (e.g., lithium, calcium, magnesium or heparin) is an acceptable alternative. Sodium is stable for at least 24 hours at room temperature and 2 weeks when refrigerated.[1, 2]

MATERIALS REQUIRED BUT NOT PROVIDED
1. Spectrophotometer.
2. Centrifuge.
3. Test tubes/rack.

PROCEDURE
Filtrate Preparation:
1. Label test tubes: blank, standard, control, patient, etc.
2. Pipette 1.0 ml of Filtrate Reagent to all tubes.
3. Add 50 µl of sample to all tubes and distilled water to the blank.
4. **Shake all tubes vigorously and mix continuously for 3 minutes.**
5. Centrifuge tubes at high speed (1,500G) for 10 minutes and test the supernatant fluids as described below, taking care not to disturb the protein precipitate.

COLOR DEVELOPMENT
1. Label test tubes corresponding to the above Filtrate tubes.
2. Pipette 1.0 ml Acid Reagent to all tubes.
3. Add 50 µl of Supernatant to respective tubes and mix.
4. Add 50 µl of Color Reagent to all tubes and mix.
5. Zero spectrophotometer with distilled water at 550 nm.
6. Read and record absorbance of all tubes.

NOTE: The chemistry reaction of this procedure involves a reduction in absorbance, as opposed to the usual absorbance increase. The absorbance of blank should be higher than the test samples.

CALCULATIONS

Abs. = Absorbance
S = Sample
STD = Standard

$$\frac{\text{(Abs. of Blank - Abs. of S)}}{\text{(Abs. of Blank - Abs. of STD)}} \times \text{Conc. of STD (mEq/L)} = \text{Conc. of S (mEq/L)}$$

SAMPLE CALCULATION

Assume the Standard with a sodium value of 150 mEq/L, gave an absorbance of 0.803 while the Sample and the Blank had absorbances of 0.880 and 1.406 respectively. The sodium concentration of the Sample may then be calculated as follows:

$$\frac{(1.406 - 0.880)}{(1.406 - 0.803)} \times 150 = \frac{0.526}{0.603} \times 150 = 131 \text{ mEq/L}$$

PROCEDURAL LIMITATIONS

1. When preparing filtrates, inadequate shaking or centrifugation will cause falsely lowered test results.
2. Blood calcium, chloride, and potassium levels of up to 3 times normal reportedly exert no adverse influence on the procedure; phosphorus levels exceeding 5 times normal likewise present no problems.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material with established sodium values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES[1, 2]

135 - 155 mEq/L

PERFORMANCE CHARACTERISTICS

1. Linearity: 200 mEq/L
2. Sensitivity: Based on an instrument resolution of A = 0.001, the present method has a sensitivity of 0.5 mEq/L.
3. Comparison: A comparison between this procedure and flame photometric analysis produced a regression equation of Y = 0.69X + 4.5 with a coefficient of correlation of 0.92.
4. Precision Study:

	Within Run	
Mean (mEq/L)	S.D.	C.V.%
146	7	5
127	4	3

	Run to Run	
Mean (mEq/L)	S.D.	C.V.%
148	5	4
139	14	10

REFERENCES

1. Tietz, N.W., *Fundamentals of Clinical Chemistry*, W.B. Saunders Co., Phila, PA, p. 874.
2. Henry, R.F., et al., *Clinical Chemistry Principles and Technics*, 2nd Ed., Harper and Row, Hagerstein, M.D., (1974).
3. Maruna, RFL, *Clin. Chem Acta*, 2:581, (1958).
4. Trinder, P: *Analyst*, 76:596, (1951).

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