

ANNEX 3

Method for measuring urinary iodine using ammonium persulfate (method A)

A3.1 Principle

Urine is digested with ammonium persulfate. Iodide is the catalyst in the reduction of ceric ammonium sulfate (yellow) to the cerous form (colourless), and is detected by the rate of colour disappearance (Sandell-Kolthoff reaction).

A3.2 Equipment

Heating block (vented fume hood not necessary), colorimeter, thermometer, test tubes (13 x 100 mm), reagent flasks and bottles, pipettes, balance scales.

A3.3 Reagents

1. Ammonium persulfate (analytical grade)
2. As_2O_3
3. NaCl
4. H_2SO_4
5. $\text{Ce}(\text{NH}_4)_4(\text{SO}_4)_4 \cdot 2\text{H}_2\text{O}$
6. Deionized H_2O
7. KIO_3

A3.4 Solutions

1.0 M Ammonium persulfate: Dissolve 114.1 g $\text{H}_2\text{N}_2\text{O}_8\text{S}_2$ in H_2O ; make up to 500 ml with H_2O . Store away from light. The solution is stable for at least one month.

5 N H_2SO_4 : Slowly add 139 ml concentrated (36 N) H_2SO_4 to about 700 ml deionized water (careful – this generates heat!). When cool, adjust with deionized water to a final volume of 1 litre.

Arsenious acid solution: In a 2000 ml Erlenmeyer flask, place 20 g As_2O_3 and 50 g NaCl , then slowly add 400 ml 5 N H_2SO_4 . Add water to about 1 litre, heat gently to dissolve, cool to room temperature, dilute with water to 2 litres, filter, and store in a dark bottle away from light at room temperature. The solution is stable for months.

Ceric ammonium sulfate solution: Dissolve 48 g ceric ammonium sulfate in 1 litre 3.5 N H₂SO₄. (The 3.5 N H₂SO₄ is made by slowly adding 97 ml concentrated (36 N) H₂SO₄ to about 800 ml deionized water (careful, this generates heat!) and when cool, adjusting with deionized water to a final volume of 1 litre). Store in a dark bottle away from light at room temperature. The solution is stable for months.

Standard iodine solution, 1 µg iodine/ml (7.9 µmol/l): Dissolve 0.168 mg KIO₃ in deionized water to a final volume of 100 ml (1.68 mg KIO₃ contains 1.0 mg iodine; KIO₃ is preferred over KI because it is more stable, but KI has been used by some laboratories without apparent problems). It may be more convenient to make a more concentrated solution, e.g., 10 or 100 mg iodine/ml, then dilute to 1 µg/ml. Store in a dark bottle. The solution is stable for months. Useful standards are 20, 50, 100, 150, 200, and 300 µg/l.

A3.5 Procedure

1. Mix urine to suspend sediment.
2. Pipette 250 µl of each urine sample into a 13 x 100 mm test tube. Pipette each iodine standard into a test tube, and then add H₂O as needed to make a final volume of 250 µl. Duplicate iodine standards and a set of internal urine standards should be included in each assay.
3. Add 1 ml 1.0 M ammonium persulfate to each tube.
4. Heat all tubes for 60 minutes at 100 °C.
5. Cool tubes to room temperature.
6. Add 2.5 ml arsenious acid solution. Mix by inversion or vortex. Let stand for 15 minutes.
7. Add 300 µl of ceric ammonium sulfate solution to each tube (quickly mixing) at 15 to 30-second intervals between successive tubes. A stopwatch should be used for this. With practice, a 15-second interval is convenient.
8. Allow to sit at room temperature. Exactly 30 minutes after the addition of ceric ammonium sulfate to the first tube, read its absorbance at 420 nm. Read successive tubes at the same interval as when adding the ceric ammonium sulfate.

A3.6 Calculation of results

Construct a standard curve on graph paper by plotting iodine concentration of each standard on the abscissa against its optical density at 405 µg/l (OD₄₀₅) on the ordinate.

A3.7 Notes

1. This is modified from the former method (27) substituting ammonium persulfate for chloric acid (more toxic) as the digestant.
2. Since the digestion procedure has no specific end-point, it is essential to run blanks and standards with each assay to allow for variations in heating time, etc.
3. The exact temperature, heating time, and cooling time may vary. However, within each assay, the interval between the time of addition of ceric ammonium sulfate and the time of the reading must be the same for all samples, standards, and blanks.
4. With the longer ceric ammonium sulfate incubation and with 15-second interval additions of CAS, up to 120 tubes can be read in a single assay.
5. The volumes and proportions of samples and reagents can be varied to achieve different concentrations or a different curve shape, if conditions warrant. If different tube sizes are used, corresponding sized holes in the heating block are also needed.
6. If necessary, this method could probably be applied without a heating block, using a water, oil, or sand bath, but this is not recommended. It is essential that all tubes be uniformly heated and that the temperature be constant within the range described above.
7. Test tubes can be reused if they are carefully washed to eliminate any iodine contamination.
8. Various steps of this procedure are suitable for automation. For example, the colorimetric readings can be done in microtiter plates with a scanner, and the standard curves plotted and read on a simple desk computer.