Method for estimation of iodine in urine

Described herewith is the standard operating procedure for estimation of iodine in urine by colorimetric method after chloric acid digestion

A) **Principle**: Urine is digested with chloric acid under mild conditions and iodine is determined manually by its catalytic role in the reduction of ceric ammonium sulfate in the presence of arsenious acid. As the reduction proceeds the intensity of color decreases and this can be readily measured in a spectrophotometer at 420 nm. The method is fast and inexpensive, and the digestion is less harsh than some other methods. This method can measure urinary iodine concentrations in the range of 0-150 mcg/liter but can be extended further to cover a wider range of values.

B) Equipment's and materials required

1. Oven with fan exhaust,
2. Vented fume hood on oven for perchloric acid escape,
3. UV spectrophotometer,
4. Thermometer,
5. Timer (stop watch reliable to 5 second),
6. Test tubes (15mm x 100mm),
7. Funnel (56x100 mm)
8. Reagent flasks and bottles,
9. Glass Pipettes
10. Micropipettes
11. Whatman no 1 filter paper
12. Laboratory balance.

C) Chemicals (analytical grade AR /GR)

a. KCLO3 (potassium chlorate),
b. HCLO4 (perchloric acid, 70%)
c. As2O3 (arsenic trioxide),
d. NaOH (sodium hydroxide),

e. H_2SO_4 (sulfuric acid)

f. $Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$ (ceric ammonium sulfate),

g. KIO_3 (potassium iodate),

h. HCL (Hydrochloric Acid)

i. Double distilled water (free of iodine and other contaminants)

D) Preparation of reagents

1) Chloric acid solution:

In a 2000ml Erlenmeyer flask, 500g potassium chlorate is dissolved in 910ml hot double distilled water until the soluble state (normally a little amount remains undissolved). 375 ml of 70% perchloric acid is added drop wise (approx. 15 ml/min) while stirring constantly. This preparation is carried out in a vented fume hood as it produces toxic fumes. Subsequently, the solution is kept in a freezer of refrigerator overnight for better separation. The next day it is filtered through a filter paper, (Whatman # 1) and stored in a refrigerator at 4^0C.

2) Arsenious Acid Solution:

0.986 g arsenic trioxide is taken in a 1000 ml volumetric flask and is dissolved in 10 ml of 0.5 N hot sodium hydroxide. This solution is transferred into 750 ml chilled double distilled water. Then 20 ml concentrated HCL and 39.6 ml conc. sulphuric acid (98%) is added drop wise with constant mixing. The solution is stored in amber color bottle at room temperature.

(The solution is stable for months).

3) Sulphuric Acid Solution (3.5N H_2SO_4):

97 ml concentrated sulfuric acid (98%) is added drop wise into 800 ml chilled double distilled water (carefully as this generates heat) and final volume is made up to 1 liter with double distilled water.

4) Ceric ammonium sulfate solution:

48g ceric ammonium sulfate is dissolved in 1 liter of 3.5N H_2SO_4. This is stored in an amber color bottle at room temperature. (The solution is stable for months).

5) Stock Iodine Standard (1mg/ml): 168.5 mg KIO_3 is dissolved in double distilled water to make a final volume of 100 ml. This is stored in an amber color bottled (This solution is stable for months).

6) Dilute Iodine Standard (1ug/ml): Take 100 ul of Stock Iodine Standard and make a volume to 100 ml with double distilled water.

7) Working Iodine Standard: Make the following serial dilutions from diluted Iodine Standard (1ug/ml) into volumetric flasks (10 ml) with double distilled water (diluent). These dilutions are made freshly.

ug/dl	Dilution factors
5 ug	: 0.5 ml of 1 ug/ml standard + 9.5 ml diluent
10 ug	: 1.0 ml of 1 ug/ml standard + 9.0 ml diluent
15 ug	: 1.5 ml of 1 ug/ml standard + 8.5 ml diluent
20 ug	: 2.0 ml of 1 ug/ml standard + 8.0 ml diluent

E) Methodology for testing

Step I.: Preparation of standard and samples

1. The urine sample is shaken to evenly suspend any sediment.
2. 250 ul of each urine sample is pipetted into a 15x100 mm test tube.
3. Iodine standards are prepared from the 1 ug/ml stock iodine solution.
4. The iodine standards corresponding to 0/5/10/15 and 20 ug/dl are prepared.

Step II. Digestion

1. 750 micro liter of chloric acid solution is added to each tube (samples, blank, internal quality control sample, standards) and mixed gently.

2. All tubes are placed in the oven at 110^0C-120^0C for 75 minutes (with a fume hood for the trapping of perchloric acid).

3. There will be very little volume change during heating. Some samples may be faintly yellow.

4. All the tubes are cooled at room temperature for 15 minutes. Then, the decreased volume is adjusted with double distilled water to their original volume (1.0 ml) and vortexed.

Step III:

3.5 ml of Arsenious Acid is added to each test tube and after mixing all test tubes were kept for 15 minutes at room temperature.

Step IV :

350 microliter of ceric ammonium sulfate solution is added at a fixed interval of time to each tube and quickly mixed with help of a vortex. A stopwatch is used to keep a constant interval between additions to successive tubes, (30 seconds was a convenient interval). Exactly 20 minutes after addition of ceric ammonium sulfate to the first tube, the reduction is read spectrophotometrically at 420 nm against the reagent blank at the same interval. (Successive tubes were arranged in a such a manner that the interval between the time of addition of ceric ammonium sulfate and the time of the reading is the exactly 20 minutes for all samples, standards and blanks).

Step 5: Calculation of results: The exact value of urine sample's iodine is calculated as follows:

1. The average absorbance value for each set of reference standard, control and samples is calculated.

2. A standard curve is constructed by plotting the mean absorbance obtained for each reference standard against its concentration micro g/dl on linear graph paper, with absorbance on the vertical (Y) axis and concentration (micro g / dl) on the horizontal (X) axis.

F. PRECAUTIONS:

1. Since the digestion procedure has no specific end point, it is essential to run blanks and IODINE standards with each assay to allow for variations in heating time, etc.

2. The exact temperature, heating time and cooling time can 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.

3. In this procedure it is convenient to run 60 sample's tubes per assay of which 5 are standards (at concentrations of 0/5/10/15and 20 mcg/dl).

4. Perchloric acid fumes can be toxic and the complex generated may be harmful, particularly if allowed to dry in a ventilation system. The recommended method releases much less perchloric acid than other digestion methods.

5. The exact time and temperature is not critical as long as all tubes are heated the same way.

6. 1.68mg KIO3 contains 1 mg iodine KIO3 is preferred over KI because it is more stable.

7. Test tubes can be reused if they are carefully washed to eliminate any iodine contamination.

8. Separate pipettes should be used for all the test tubes and also pipettes used for preparation of each standard solution should be kept separately and not be mixed with the general pool of glasswares. They should be kept separately for all times to avoid contamination.

G: General Instruction and Precautions

Preparation of Chromic Acid for washing glassware

Saturated solution of K_2 Cr_2 0_7 (28 gm) is prepared in 500 ml of distilled water. Add 500 ml of Technical/Commercial grade conc. H_2SO_4 slowly (if solution of potassium dichromate is in chilled condition, heat evolved will be less). Discard chromic acid if it has turned green/light green.

i) Dip the glass ware for 24 hours in chromic acid

ii) Use gloves to take out glassware from chromic acid and then wash repeatedly with tap water. Rinse twice with distilled water and finally double distilled water.

iii) Dry at 80-1000 C in oven

H. Precautions to be taken during preparation of reagents and carrying out the assay

i) Keep all the glassware and tips used for preparation of standards separately and wash them also separately.

I. Pooled Sample:

Once the method is standardized prepare the urine for internal quality assessment (Use pooled sample, mixed properly, or collect from one individual about 250-300

ml). Analyze the sample 20-25 times with standard and blank in duplicate. Calculate mean and standard deviation. (Consult statistician/expert who can do this). The value of this sample should be between 5-15 ug/dl. If not collect fresh sample or dilute the sample with double distilled water to get proper range. Store, this sample in small aliquots of approximately 1.0 ml (100 to 150 aliquots) at in refrigerator (4-80C) and analyze one aliquot with every batch of unknown samples.

For analysis of the unknown samples, use the batch of 40 tubes. (Two tubes for blank, 4 standards (5, 10, 15, 20 ug/dl), one internal quality assessment sample and once in three weeks one external quality assessment sample + remaining unknown samples).

Result of internal quality assessment sample is used for drawing Levy - Jennings Plot.