

Components

- A. Protocol D1 instructions for thiocyanate analysis of urine.
- B. Thirty (30) flat-bottomed plastic bottles with screw capped lids.
- C. Three (3) graduated 1 ml plastic pipettes.
- D. Ten (10) white **standard** papers with thiocyanate equal to 10 ppm.
- E. One hundred (100) yellow **indicator** papers glued to strips of clear plastic. STORE IN FREEZER. Stable for one month only at room temperature.
- F. Colour chart with ten (10) shades of colour which correspond to 0-100 ppm thiocyanate.
- G. Bottle containing 0.5 g potassium permanganate.

Method (Complete steps 4 to 7 quickly as the enzyme acts rapidly to release HCN)

1. Urine samples should either be fresh or stored in the freezer for up to 6 months.
 2. Prepare a sulphuric acid solution, add 5.5 ml of concentrated (96%) sulphuric acid to 100 ml water in a beaker slowly with stirring. TAKE CARE! heat is produced do not add water to acid!
 3. Prepare a permanganate solution by dissolving 100 mg potassium permanganate in 5 ml water. This is stable for 2 months if stored at room temperature and away from direct sunlight.
 4. Follow sketch 1. Use plastic pipette to add 1.0 ml urine to a flat-bottomed plastic bottle followed by three (3) drops of sulphuric acid solution from a second plastic pipette and mix.
 5. Add three (3) drops of permanganate solution from a third plastic pipette and mix gently.
 6. IMMEDIATELY add a yellow **indicator** paper attached to a plastic strip so that the paper does not touch the liquid in the bottle. When not in use STORE INDICATOR PAPERS IN FREEZER.
 7. IMMEDIATELY close the bottle with a screw capped lid. The magenta colour should disappear.
 8. A positive and negative control should be run for each set of experiments.
 - a. For a negative control, prepare another sample as shown in sketch 1 but with 1.0 ml water instead of urine.
 - b. For a positive control follow sketch 2. Place a white **standard** paper disc in the bottle. Add 1.0 ml water, three drops of sulphuric acid solution, mix and add three drops of permanganate solution. IMMEDIATELY add the yellow **indicator** paper and IMMEDIATELY close the bottle with a screw capped lid and carefully mix to avoid wetting the paper. The magenta colour may remain for some time. (wash the pipettes thoroughly to remove urine, sulphuric acid and permanganate).
 9. Allow the bottles to stand for 16-24 hr at room temperature.
 10. Open the bottles and match the colour of the **indicator** papers against the shades of colour on the colour chart supplied.
 11. Read from the colour chart the thiocyanate content in ppm in the urine sample (see 16b). Check that the negative control is zero and that the positive control gives a colour of about 10 ppm.
- THIS SECTION TO BE FOLLOWED IF YOU HAVE A SPECTROPHOTOMETER**
12. For each sample, carefully remove the plastic backing sheet from the **indicator** paper.
 13. Place the paper in a test tube and add 5.0 ml of water measured accurately.
 14. Leave the test tube at room temperature for about 30 min with occasional gentle stirring.
 15. Measure the absorbance at 510 nm of the solution, subtract the value of the negative control.
 16. The thiocyanate content in ppm is calculated by the equation¹
 - a. thiocyanate content (ppm) = 78 x absorbance
 - b. thiocyanate content in $\mu\text{mol/Lt}$ = thiocyanate content (ppm) x 17.2
 17. The thiocyanate content obtained for the same sample of urine, from both measurements 11 and 16 should be about the same. Also check the **standard** value agrees using both methods.

Troubleshooting

The thiocyanate content of the white **standard** paper should be about 10 ppm. Possible problems could be:

- If the **indicator** paper is left at room temperature it gradually becomes darker and after one month its colour will be around 1-2 ppm on the colour chart.
- If the **indicator** paper has been left in bright sunlight it becomes bleached on one side and is no good.
- Permanganate solution has decomposed which would give a low result. Make up a new solution.
- Sulphuric acid solution was not made up properly or may be too dilute. Make up a new solution.
- If you use a bottle which is not gas tight (e.g. the screw cap is cracked) then gas could escape and this would give a low result.
- As stated in steps 6 & 7, it is important to add the **indicator** paper immediately after the permanganate and then immediately close the lid otherwise there will be a loss of gas which would give a low result.

Reference

¹Haque, R. and Bradbury, J. H. (1999) Simple kit method for determination of thiocyanate in urine. *Clinical Chemistry*, 45, 1459-1464.

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