1. Urine samples should either be fresh or samples may be used which have been stored in the deep freeze of a refrigerator. In the deep freeze urine samples are stable for periods of at least 6 months.

2. Prepare a sulphuric acid solution by adding 5.5 mL of concentrated, about 96% sulphuric acid, to 100 mL of water in a beaker, slowly and with stirring. Take care, heat is produced; do not add the water to the sulphuric acid.

3. A potassium permanganate solution is prepared by weighing out 100 mg of potassium permanganate and dissolving in 5 mL of water. This solution is stable for more than 2 months if stored at room temperature away from direct sunlight.

4. Please follow sketch 1. Using the plastic pipette, 1.0 mL urine is placed in a flat bottomed plastic bottle followed by three drops of sulphuric acid from the second plastic pipette and the solution is mixed.

5. Three drops of potassium permanganate solution are added from the third plastic pipette.

6. IMMEDIATELY add a yellow picrate paper attached to a plastic strip (sketch 1). The picrate paper must not touch the liquid in the bottle. WHEN NOT IN USE STORE PICRATE PAPERS IN THE DEEP FREEZE OF THE REFRIGERATOR.

7. IMMEDIATELY close the bottle with a screw capped lid and gently mix the solution. The magenta permanganate colour should disappear almost immediately.

8. Prepare another sample as in sketch 1 but with no urine and 1 mL of water instead of the urine as a blank.

9. Every time that you run a set of thiocyanate experiments, it is important to check the method by running a standard (4 ppm or 40 ppm).

10. Please follow sketch 2. As a standard, place a paper disc loaded with thiocyanate in a bottle, add 1.0 mL water, 3 drops of sulphuric acid, mix and add 3 drops of potassium permanganate solution (see sketch 2). IMMEDIATELY add a yellow picrate paper and IMMEDIATELY close the bottle with a screw cap lid and then gently mix the solution. The permanganate colour may remain for some time. (Wash the different plastic pipettes thoroughly with water to remove urine, sulphuric acid and permanganate.)

11. Allow the bottles to stand for 16-24 hour at room temperature.

12. Open the bottles and match the colour of the picrate papers against the shades of colour of the colour chart supplied.

13. Read off from the colour chart, the thiocyanate content in ppm in the urine sample. Also check that the blank is zero and the standard gives a colour of about 4 ppm or 40 ppm depending on which standard paper you used.

**FOLLOW THIS SECTION IF YOU HAVE A SPECTROPHOTOMETER**

14. Carefully remove the plastic backing strip from the picrate paper. The plastic strip may be washed and used again.

15. Place the picrate paper in a test tube and add 5.0 mL of water measured accurately with a pipette.

16. Leave the test tube at room temperature for about 30 min, with occasional gentle stirring.

17. Take the blank picrate paper (see 8 above), remove its plastic strip and place the yellow picrate paper in 5.0 mL of water for about 30 min with occasional gentle stirring.

18. Measure the absorbance at 510 nm of the picrate solution from 16 against the blank from 17.

19. The thiocyanate content in ppm is calculated by the equation:  
   \[
   \text{thiocyanate content (ppm)} = 78 \times \text{absorbance}
   \]
   Thiocyanate content in µ mol/L = thiocyanate content (ppm) x 17.2.

20. The thiocyanate content obtained for the same sample of urine, from both measurements 13 and 19, should be about the same. Also check that the standard value from 10 is about the same using both methods and is about 4 or 40 ppm.
FOOTNOTE –IF YOU WISH TO PREPARE YOUR OWN PICRATE PAPERS

To prepare your own picrate papers you need your own bottle of moist picric acid purchased from BDH or another supplier. Weigh out 1.4 g of moist picric acid and add 100 ml of sodium carbonate solution, made by dissolving 2.5 g of sodium carbonate in 100 ml of water. Using a filter paper sheet supplied in the kit, cut about a 10 cm x 10 cm square of paper and place it in the yellow picrate solution in a dish for about 20 sec and hang it up to dry in air. Note. Wear gloves if available when handling picric acid papers. Wash off with water any yellow picric acid on hands. Unevenly coloured sections of the paper particularly at the edges are cut off. The paper is cut into 30 mm x 10 mm rectangular pieces. Each piece is glued using one small drop of PVA hobby glue to a plastic strip (10 mm x 50 mm), cut from overhead transparency plastic sheet supplied in the kit. It is glued so that the upper end of the yellow paper is 5-10 mm from one end of the plastic strip (see sketch 1 or 2). Picrate papers must not be left in bright sunlight and should not be left in laboratory light for long periods. STORE PICRATE PAPERS IN THE DARK IN THE DEEP FREEZE OF THE REFRIGERATOR WHERE THEY ARE STABLE INDEFINITELY. At room temperature they gradually darken and after one month cannot be used with the colour chart but may still be used with the spectrometer method, because the darker colour cancels out.

TROUBLE SHOOTING

When using the thiocyanate standard samples (4 and 40 ppm) then the result should not be very different from 4 or 40 ppm. If the difference is large then there is something wrong. Possible problems could be:

(1) If the picrate paper has been left at room temperature for more than one month then it will gradually become darker, and will look like about 1-2 ppm on the colour chart. If the picrate paper has been left in bright sunlight it will become bleached on one side and will be spoiled.

(2) Permanganate solution has decomposed. This would give a low result.

(3) Sulphuric acid solution was not made up properly and is too dilute. This could give a low result.

(4) Use of a bottle which is not gas tight (e.g. screw cap is cracked), would allow HCN gas to escape and give a low result.

LIST OF COMPONENTS OF KIT D 1

The kit has the following components:

1. Protocol D 1, which gives full instructions for thiocyanate analysis of urine.
2. 30 flat-bottomed plastic bottles with screw lids.
3. Three graduated 1 ml, plastic pipettes.
4. Ten standard papers, which contain thiocyanate equal to 4 ppm and ten standards equal to 40 ppm.
5. 100 yellow picrate papers glued to strips of clear plastic with hobby glue. STORE IN THE DEEP FREEZE OF REFRIGERATOR. STABLE FOR ONE MONTH ONLY AT ROOM TEMPERATURE.
6. Colour chart with 10 shades of colour from yellow to brown which correspond to 0-100 ppm thiocyanate.
7. Bottle containing potassium permanganate. You must supply the sulphuric acid.
8. Filter paper and plastic overhead transparency sheets for making more picrate papers. You must supply the picric acid, which cannot be sent by air.

References

1. Picrate paper
2. 3 Drops Potassium Permanganate
3. 3 Drops Sulphuric acid
4. 1.0mL Urine

Bottle

2. Picrate paper
3. 3 Drops Potassium Permanganate
4. 3 Drops Sulphuric acid
5. 1.0mL Water
6. Thiocyanate standard paper

Bottle