

## **A Procedure for Detection of Non-Protein Nitrogen Containing Substances in Milk**

A step-wise procedure for detection of non-protein nitrogen containing substances in milk is given below. This procedure will provide approximate amount of non-protein nitrogen rich substances which have been mixed with milk as adulterant. The procedure is based on the determination of total protein in milk directly in Milkoscan (Milkoscan gives the values of total protein in milk directly), and estimation of total nitrogen in the milk sample (which may include nitrogen of protein and non-protein substances present in milk), which is then converted into the equivalent amount of protein by using the accepted conversion factor, and then comparing these two values of protein so obtained.

The procedure is as follows:

- A. Take a sample of milk (reconstituted milk in case of milk powder) and divide it into two parts.
- B. Analyse the Part 1 of the sample in Milkoscan for total protein content. The result would give the total protein (milk proteins as well as non-milk proteins, if mixed with milk). The test method is provided in Annex 1.
- C. Analyse Part 2 of the sample through *Kjeldahl* method for total nitrogen content. The nitrogen content so obtained would include nitrogen from all the sources – protein and non-protein. Convert the nitrogen content into protein content by using the approved conversion factor of 6.38. The standard AOAC method to be used is provided in Annex 2.
- D. Compare the protein content obtained directly in Step B with the protein content obtained in Step C. If the protein content obtained through Step C is appreciably higher than that analyzed in Step B then it is an indication that the milk has been adulterated with non-protein nitrogen rich substance, which could be urea, melamine or other sources.

If the difference in the values of proteins obtained in Step B and Step C is not substantial then there is no indication of adulteration of milk with non-protein nitrogen substances. Small differences could be due to acceptable variations inherent in the analysis by these methods.

Notes:

1. It is important that the Milkoscan has been calibrated with pure, unadulterated milk using *Kjeldahl* method.
2. Theoretically, the above suggested procedure will be able to detect non-protein nitrogen from melamine content above 0.1% in milk. (0.1% of melamine in milk will translate into a difference of 0.4% protein as determined by *Kjeldahl* method and using Milkoscan.)
3. The test method using Milkoscan provided in Annex 1 is for guidance only. The exact procedure for using particular model of Milkoscan provided in the manufacturer supplied operation manual should be used.