



The tasks of the European Union Reference Laboratories (EURLs, former CRLs) are laid down in Regulation (EC) No 882/2004 of the European Parliament and of the Council and following amendments [1]. The main objective of the EU-RL for Chemical Elements in Food of Animal Origin (EU-RL CEFAO) is to improve the quality, accuracy and comparability of the results produced by EU National Reference Laboratories (NRLs), i.e. through the preparation of ad-hoc reference materials for Proficiency Tests (PTs). This work describes the preparation of liquid milk used as testing material in EU-RL CEFAO PTs in the five-year period 2006 -2011. The material was prepared according to the EU-RL CEFAO procedure as described below.



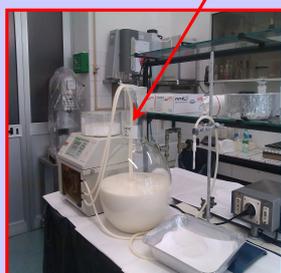
1. **SAMPLING**  
About twenty litres of commercial long-life partially skimmed cow milk, previously analyzed for the endogenous content of the analytes, were chosen as test material

2. **SPIKING**  
Standard solutions of As, Cd and Pb were gravimetrically prepared and quantitatively added to the milk previously weighted.



3. **HOMOGENIZATION**  
Magnetic stirring for 30'

#### 4. DISPENSING



In the phase of dispensing two peristaltic pumps were used (one for milk dispensing and the other for maintaining the homogenization of the bulk material)



Each vial was singly numbered

#### 8. STABILITY

The stability of material was checked for As, Cd and Pb in order to evidence that the test material would have sufficient stability over the course of the exercise. Subsequently, a longer stability was tested on different batches after 7, 18 and 30 months. Vials containing the sample were stored in darkness at room temperature and the level of concentration found was compared with the assigned value ("consensus" value) from the relevant PT.

For each analyte, the material resulted stable for 18 months as shown in the table.

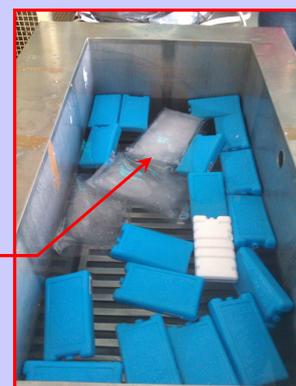


#### 5. STERILIZATION

In order to ensure the milk stability, the sample was sterilized in autoclave for 3' at 120°C

#### 6. QUENCHING

The vials were quenched in chilled water to avoid the browning process of sugars present in milk



#### 7. HOMOGENEITY

Homogeneity of material is tested according to the Harmonized Protocol [3].  $(\sqrt{n})-1$  vials, where n is the total number of items produced, are randomly chosen and the test items are analysed in duplicate. The content of the analytes is assessed by means of an accredited method.

### INTERNAL QUALITY CONTROL (IQC)

The selection of proper materials to be used both in method validation and in IQC is a critical point for laboratories dealing with the analyses of substances having a maximum level [2]. In the field of chemical elements in food of animal origin, Certified Reference Materials (CRMs) are lacking in the relevant matrices and/or analytes of interest at a suitable level. Furthermore, samples submitted to control are usually in a different physical state from freeze-dried, which is the most common state of the CRMs.

A surplus of test items is distributed by EU-RL CEFAO to the participants of its circuit. This extra material can be used for validation/IQC purpose since a reference value of concentration is set as assigned value based on the consensus approach.

Element	13 <sup>th</sup> PT Assigned value	Stability test value (7 months)	12 <sup>th</sup> PT Assigned value	Stability test value (18 months)	11 <sup>th</sup> PT Assigned value	Stability test value (30 months)
As (mg/kg)	0.078	0.076	0.125	0.122	0.051	0.051
Cd (mg/kg)	0.0072	0.0070	0.006	0.005	0.007	0.002
Pb (mg/kg)	0.035	0.036	0.027	0.030	0.014	0.010

### CONCLUSIONS

The results obtained from the stability test at low levels suggest that the behaviour of spiked chemical elements is similar to the one of endogenous analytes. The procedure developed by EU-RL CEFAO for the production of spiked milk is suitable not only for obtaining test materials for PT but also for producing samples for Internal Quality Control.

#### References

- [1] Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules and following amendments.  
[2] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs and following amendments  
[3] The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, M. Thompson, S. R. L. Ellison, R. Wood, *Pure and Appl. Chem.* 2006, 78(1), 145-196