The BCR agro-food analysis programme – A systematic approach to certification exemplified by studies on aflatoxin M_1 in milk powder and toxic and nutritional elements in meats

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Summary. The Community Bureau of Reference (BCR) has undertaken the development of a range of reference materials designed to meet the principal needs for food analysis and measurement. This work falls within the scope of a general collaborative programme designed to improve measurement accuracy and comparability within Europe.

The development of well characterised food reference materials frequently poses serious difficulties, not only with respect to stability and homogeneity, but also for accurate certification. A pre-requisite for accurate certification is a high level of agreement between the collaborating laboratories, preferably when employing methods based on different principles.

Especially for trace organic analysis, it is necessary to carry-out preliminary intercomparisons of candidate certification methods in order to identify and eliminate the major sources of error. This process is greatly facilitated if the critical steps such as recovery, clean-up and final determination can be studied separately. The paper describes this approach by reference to three milk-powders which were recently certified for their aflatoxin M_1 content and presents results for bovine muscle, bovine liver and pig kidney CRMs recently certified for toxic and nutritional element.

1 Introduction

The sector of the BCR programme concerned with agro-food is responding to the recognition by industrial, public health and nutritional laboratories that reliable measurement of many of the most important properties cannot be achieved through reliance on written standards alone.

Requirements of GLP, the demands imposed by laboratory accreditation and the increased awareness of the needs for in-house quality assurance schemes have caused laboratories to seek means by which they can check the reliability of their own measurements by external comparison. Such comparison is commonly made by participation in ring-tests or, more conveniently, by use of certified reference materials (CRMs), when available.

The enormity of the problem of satisfying the needs of those concerned with agro-food measurements is well reflected by the fact that there are at least 4000 individually documented methods of measurements in this field alone.

2 Objectives of the BCR programme

The objective of the programme is to improve the comparability and accuracy of measurements through the organisation of carefully designed intercomparisons involving experienced European Laboratories. The intercomparisons are designed both to establish the "state of the art" for the particular measurement and to identify the principal sources of error, be they due to an inherent weakness in a method or to its poor application.

The intercomparions are repeated, modified as necessary, until the major sources of error are reduced to a level where between-laboratory and between-method agreement is acceptable.

When appropriate, the exercise may lead to the establishment of a certified reference material, the decision depending upon the expected demand from within the Community, the nature of the analytical problem and, of course, the practicality and expense of production.

3 Current activities in the agro-food area

Some of the activities currently underway (or recently completed) in the agro-food area are summarised in Table 1.

During the past three years, 19 intercomparisons have been carried out in this sector alone, involving the collaboration of some 120 laboratories, and leading to the establishment of 8 CRMs certified for approximately 60 individual properties.

4 Systematic approach to measurements improvement

It is a fact of life that different people making ostensibly the same measurement will arrive at, to a greater or lesser extent, different results. This is inevitable simply because it is impossible to reproduce exactly the conditions of measurements in situations separated in place or time.

The origin of the differences may be obvious, e. g. known flaws in a method, poor calibration or lack of experience. Generally, however, the sources of error are more difficult to locate, especially when experienced laboratories are involved.

For trace organic analysis, for example, the critical steps in the analysis may be broadly summarised (assuming a representative analytical sample) as follows:

Calibration – pure and stable calibrant, linearity. Extraction – efficiency and repeatability. Clean-up –

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Table 1
Examples and recent projects concerned with agro-food measurement

Topic	Nature of activity	Status
Toxic and nutritional elements		
Milk	Completed	3 CRMs available
Meat	Completed	3 CRMs available
Cereals	Int. and CRM	CRMs avail. end 86
Single cell protein	Int. and CRM	CRMs avail. end 86
Trace organic analysis		
Aflatoxin M_1 in milk powder	Completed	3 CRMs available
Aflatoxin B_1 in animal feed	Int. and CRM	On-going
DON and other mycotoxins in agro-products	Int.	On-going
PCBs in fish oil	Int.	On-going
Pesticides in milk	Int.	On-going
Growth promoting hormones	Int. and CRMs	On-going
Nutritional/Composition		
Fatty acids in edible oil and fats	Int. and CRMs	CRMs avail. end 86
Major components	Int.	On-going
Fruit juice analysis	Int.	On-going
Physical properties		
Farinograph	Int. and CRMs	On-going
Water activity	CRM	Available 1987

Int. Intercomparison exercises

minimisation of losses. Final-determination – specificity in presence of coextractives, sensitivity.

Additionally, there is at each stage the ever-present risk of sample contamination, especially where concentrated or even pure calibrants are handled in close proximity to low-level samples.

In many cases, it is possible to sub-divide intercomparison exercises in a manner with allows the errors arising at the critical steps to be examined separately, thereby allowing the causes to be identified and corrected.

4.1 Aflatoxin M_1 in milk powder

This approach is clearly illustrated by the series of intercomparisons that led to the certification of three "aflatoxin M_1 in milk powder" reference materials.

In the first study, 19 laboratories made replicate measurements, using a method of their own choice, of the aflatoxin M_1 content of a milk powder (circa 0.8 µg/kg) and of a plain $CHCl_3$ solution (circa 1 µg/ml).

The results showed that even at a relatively high level of contamination, there was a wide and unacceptable between-laboratory dispersion (Fig. 1a). Further, the results for the simple chloroform solution suggested that inaccuracy of the laboratories' calibrant solutions was a major source of error.

A second study was designed which allowed separation of the sources of error, viz.:

Calibration – common aflatoxin M_1 calibrant provided + 'unkown' aflatoxin M_1 in $CHCl_3$ solution. Recovery – parallel recovery experiments on a 'blank' milk powder provided. Final determination – determination of aflatoxin M_1 in a common, 'cleaned-up' milk extract. Overall perfor-

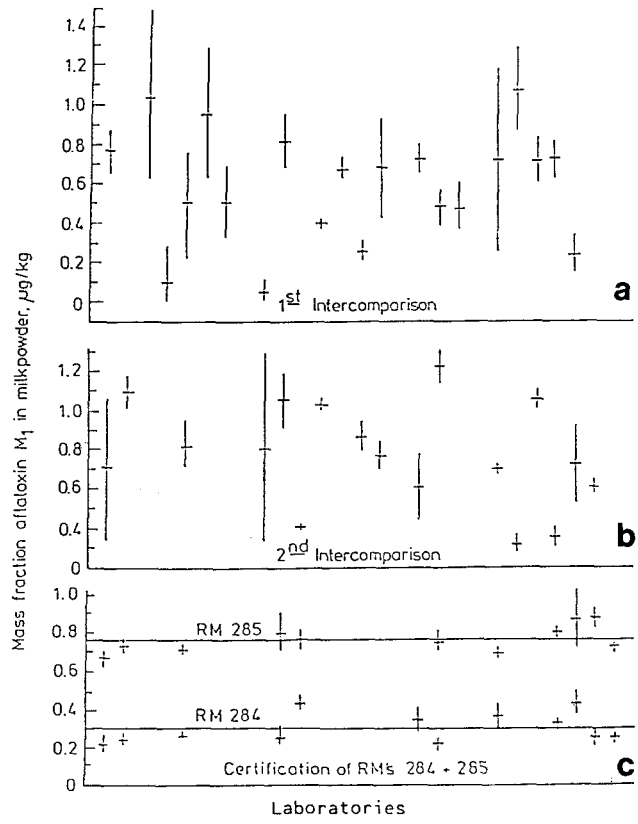

Fig. 1a–c. Comparison of results of preliminary intercomparison with those of the certification exercise (**a–c** see text)

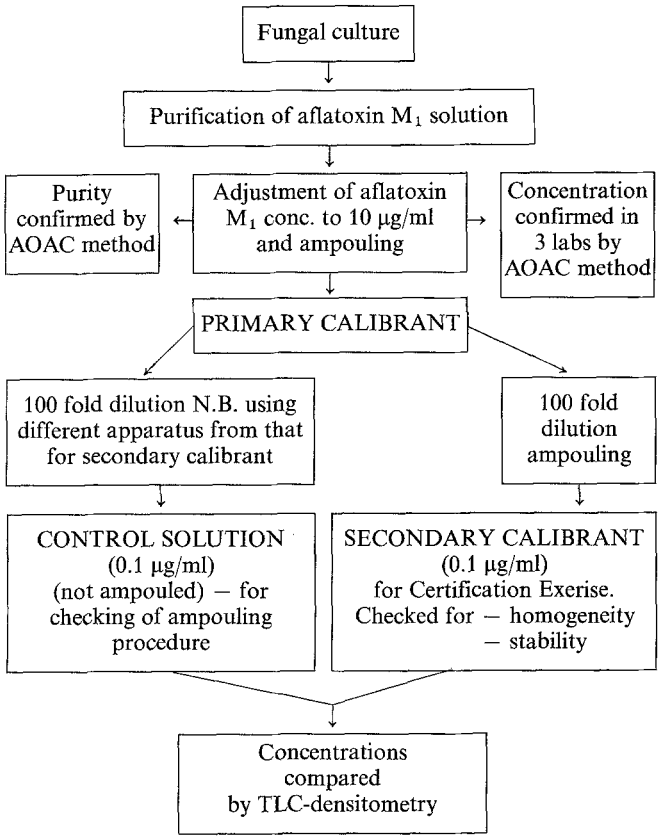

Fig. 2. Summary of procedure employed for the preparation of the aflatoxin M_1 secondary calibrant and the confirmation of its accuracy

mance – replicate measurements on an unknown milk powder.

Participants were additionally asked to correct weaknesses and errors identified in the first study for example, to ensure that excessive emulsion formation was avoided by use of procedures successfully employed in other laboratories.

The results for the unknown milk powder are presented in Fig. 1 b. Although the improvement in between-laboratory agreement was not sufficient to allow certification, the design of the study allowed the causes of error in the most divergent results to be identified, e.g., low and variable recoveries, inadequate resolution of aflatoxin M_1 in some chromatographic systems (expecially in 1-dimensional TLC); instability of aflatoxin M_1 in aqueous aceto-nitrile employed for HPLC.

Final certification of the aflatoxin content of three milk powders was planned incorporating the experience of the preliminary studies, with special attention being paid to:

– Calibration

Provision of an ampouled aflatoxin M_1 calibrant solution of demonstrated stability, having a concentration of of 0.100 ± 0.002 µg/ml as determined on the basis of measurements in three independent laboratories and the molar absorptivity data of AOAC (see Fig. 2).

– Recoveries

Supply of low-level milk powder (RM 282) for spiking and parallel recovery experiments. Demonstration that the residual aflatoxin M_1 content of RM 282 was too low to significantly influence recoveries.

Table 2. Results (ranges) of the preliminary intercomparison of methods on a bovine liver material. (The ranges of results obtained in the final certification exercise for bovine liver RM 185 are given for comparison. p = number of sets of results received

	Preliminary intercomparison		Certification exercise	
	Range of mean values	p	Range of mean values	p
Cd ng/g	200 – 1290	22	257 – 333	16
Pb ng/g	200 – 1400	22	276 – 400	17
Hg ng/g	25 – 220	6	33 – 72	9
As ng/g	7 – 30	5	23 – 50	7
Se ng/g	150 – 610	7	423 – 540	12
Cu µg/g	159 – 393	21	171 – 199	16
Zn µg/g	114 – 162	27	153 – 181	20
Fe µg/g	148 – 278	24	193 – 230	18
Mn µg/g	5 – 13	17	8.3 – 10.7	13

– Independence of measurement

Participants were required to make 8 separate measurements over a period of 3 days.

The results of the certification exercise for CRMs 284 and 285 are presented in Fig. 1 c. It is evident from Fig. 1 that a substantial improvement in the application of the various methods has been achieved.

The three CRMs, which were prepared from naturally contaminated milk, have been subjected to exhaustive homogeneity and stability tests. They are available in units of 25 g with the following certified values:

	Aflatoxin M_1 content
CRM 282	< 0.05 µg (an effective blank)
CRM 284	0.31 ± 0.06 µg/kg
CRM 285	0.76 ± 0.05 µg/kg

Full details of the project, including the preliminary intercomparisons, certification exercise and the analytical methods used have been published [1] and are given in the certification report [2], which is supplied with the CRMs. Also discussed are sources of error, together with recommendations for their control.

4.2 Toxic and nutritional elements in meat

Community legislation controls the presence of harmful substances in meat. In the case of the toxic elements, Pb, Cd, Hg and As, it has proved difficult to define convenient methods of adequate precision and accuracy, which are sufficiently robust to satisfy the needs for official reference methods.

A preliminary intercomparison of methods for a range of important elements was organised amongst experienced veterinary and food laboratories and included several laboratories specialised in the application of sophisticated methods, such as INAA and IDMS.

The conclusions of this study are summarised in the form of ranges of results in Table 2.

Detailed discussion of the results with the participants revealed a number of sources of error including: losses or contamination during sample preparation, especially where poorly controlled dry-ashing was used, application of instrumental methods below the limits of determination (e.g.,

Table 3. Certified elemental contents and uncertainties in RMs 184, 185 and 186

Element	CRM184 Bovine muscle			CRM185 Bovine liver			CRM186 Pig kidney		
	Certified value	Uncertainty	*P*	Certified value	Uncertainty	*P*	Certified value	Uncertainty	*P*
Cd	13 ng/g	± 2 ng/g	12	298 ng/g	± 25 ng/g*	13	2.71 µg/g	± 0.15 µg/g	15
Pb	239 ng/g	± 11 ng/g	11	501 ng/g	± 27 ng/g	10	306 ng/g	± 11 ng/g	12
Hg	2.6 ng/g	± 0.6 ng/g	5	44 ng/g	± 3 ng/g	6	1.97 µg/g	± 0.04 µg/g	8
As		–		24 ng/g	± 3 ng/g	5	63 ng/g	± 9 ng/g	5
Se	183 ng/g	± 12 ng/g	12	466 ng/g	± 25 ng/g	12	10.5 µg/g	± 0.6 µg/g	11
Cu	2.3 µg/g	± 0.06 µg/g	10	189 µg/g	± 4 µg/g	12	31.9 µg/g	± 0.4 µg/g	10
Zn	166 µg/g	± 3 µg/g	17	142 µg/g	± 3 µg/g	17	128 µg/g	± 3 µg/g	16
Fe	79 µg/g	± 2 µg/g	15	214 µg/g	± 5 µg/g	14	299 µg/g	± 9 µg/g	14
Mn	334 ng/g	± 28 ng/g	6	9.3 µg/g	± 0.2 µg/g	9	8.4 µg/g	± 0.3 µg/g	10

Flame AAS and ICP for Pb and Cd), inadequate preliminary treatment before final determination e.g., incomplete oxidation of organic matter with ASV methods, incomplete reduction of Se(VI) to Se(IV) before hydride generation for Se determination).

In view of the unsatisfactory nature of the results and the importance of the measurements for Community legislation and public health, three lyophilised meat reference materials were prepared. Bovine muscle, bovine liver and pig kidney were selected since it is well known that many elements accumulate preferentially in liver and kidney, whereas the levels in muscle tissue are relatively low. Thus, these three materials would provide a convenient range of concentration for many elements of interest, as well as giving some variation in matrix and "between-element" effects.

The certification exercise was carried out by a group of 28 laboratories (5 to 17 per element) and was designed so as to both eliminate problems encountered in the first study and to ensure maximum variety in methods of sample digestion and final determination. Laboratories were required to make 5 independent determinations of each element, paying special attention to choice of calibrant, calibration, use of methods that offered sufficient sensitivity and accuracy at the expected concentration and, application of a dry-weight correction as determined by a specified method.

Conclusions of particular interest for this projects include:

– There is some evidence of Pb inhomogeneity in pig kidney for sample intake of 100 mg or less. This may be connected with the observation that there are microscopic crystals of the same crystal habit as human and equine kidney stones. Analysis of samples of such stones has shown Pb contents of up to 0.1%.

– Bovine liver (CRM 185) proved more difficult to acid-digest than either the muscle or kidney materials or, indeed, than the NBS bovine liver 1577A analysed in parallel by some participants. This effect was particularly clearly demonstrated for the analyses of Cd, where unexpectedly high between-laboratory variations were recorded (within-laboratory dispersion was normal). The reason for this effect is not known but may well be connected with the complexity of liver, especially as concerns the presence of mono-, di- and tri-glycerides and, high cholesterol and phospholipid contents.

– Although satisfactory for certification, the between-laboratory agreement for Se was worse than expected, reflecting the known difficulty of accurate determination of this important element and the need to improve the methodology.

Table 4. Summary of indicative values (mass fractions) expressed on a dry weight basis for elemental contents only

Element	Bovine muscle RM 184	Bovine liver RM 185	Pig kidney RM 186
Ca µg/g	150	131	295
Cr ng/g	76 – 153	47 – 124	58 – 142
Cl mg/g	2.0	2.9	9.4
I ng/g	40	105	145
K mg/g	16.6	11.2	12.6
Mg µg/g	1020	634	829
Na mg/g	2.0	2.1	7.1
Ni ng/g	270	1400	420
P mg/g	8.3	11.7	
Major constituents %			
Total protein	86.6	63.1	74.8
Total fat	4.4	10.3	12.0
Ash	4.3	4.5	5.6
Moisture	5.3	6.2	5

The certified values and the corresponding uncertainties for the elemental contents of the three materials are summarised in Table 3.

It is of interest to note the wide range of elemental contents in the three RMs, particularly for Cd and Hg and, to a lesser extent, for Se, Cu and Mn.

Indicative values were also established for the major elements Na, K, Cl, Mg, Ca and P, generally based on measurements in three laboratories using a variety of methods. Data are also provided for I (by two laboratories using NAA with radio nuclear separation) and for Cr and Ni.

There was not considered to be sufficient information to allow certification of these elemental contents and no attempt has been made to ascribe uncertainties to them. The indicative values are presented in Table 4, together with results for the major components total protein, total fat, ash and moisture. It is suggested that these values are used in conjunction with the more detailed results given in the Certification Report [2].

The CRMs are provided in untits of circa 15 g and the recommended minimum sample intake is 200 mg for the determination of each cerfified element, with the exception

of Pb in RM 186 (pig kidney), where not less than 500 mg should be taken.

Full details of the preparation of the materials, the homogeneity and stability tests, design and evaluation of the certification exercise are given in the Certification Report [2], which is provided with the CRMs. The report also discusses principal sources of error and their control.

Acknowledgements. The success of the BCR programme relies upon the enthusiasm and skill of the many collaborating laboratories, to all of whom the BCR gives grateful acknowledgement.

References

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