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Report

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Preparation of three biological reference materials for QUASIMEME inter-laboratory testing

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Summary

Three biological materials have been prepared for IVM, Free University, Amsterdam to be used in QUASIMEME interlaboratory studies. The materials prepared are: 300 glass jars of homogenized Mediterranean mussels (QM06-1), 280 tins of homogenized blue mussels from German Bight (QO06-3) and 300 tins of homogenized Baltic herring fillets (QO06-4). The homogeneity test of the Mediterranean-mussel material (QM06-1) was performed by analysing mercury concentration and it was confirmed that the material is homogeneous.

1. Introduction

The Institute for Environmental Studies has requested Wageningen IMARES to prepare three materials for the use in QUASIMEME interlaboratory studies (ILS) under contract 06.052. The materials include a Mediterranean mussel sample (QM06-1), blue mussel sample (QO06-3) and herring sample (QO06-4). The homogeneity test was requested for Mediterranean mussel sample by duplicate analysis of mercury concentration in 10 sub-samples.

2. Objectives

- 300 glass jars of homogenized Mediterranean mussels (QM06-1) should be prepared
- Homogeneity of the QM06-1 material should be tested by duplicate analysis of mercury concentration in 10 glass jars
- 280 tins of homogenized blue mussels from the German Bight (QO06-3)
- 300 tins of homogenized Baltic herring fillets (QO06-4)

3. Materials and methods

3.1 Production of materials

The materials produced are listed in Table 1.

Table 1. Materials produced for QUASIMEME ILS.

| No. | Code | Origin and purpose | No. of lots |
|-----|--------|---|-------------|
| 1 | QM06-1 | Mussels from the Mediterranean sea (Spain) in glass jars for trace metal contaminant analysis | 300 |
| 2 | QO06-3 | Mussels from the German Bight in tins for organic contaminant analysis | 280 |
| 3 | QO06-4 | Herring from the Baltic sea in tins for organic contaminant analysis | 300 |

3.1.1 QM06-1(Mediterranean mussels)

About 120 kg of fresh mussels originating from the Mediterranean Sea (Spain) was purchased (Adri and Son) on 28 September 2006. After cooking the mussels for 5 minutes the shells were removed. The material was minced and stored at 0°C. The material was homogenised and sterilized in glass jars on 2 October 2006 according to the procedure described in section 3.1.4.

3.1.2 QO06-3 (blue mussels from the German Bight)

About 100 kg of fresh mussels originating from the German Bight was purchased on 27 September 2006. After cooking the mussels for 5 minutes the shells were removed. The material was minced and stored at 0 °C. The material was homogenised and sterilised in tins on 3 October 2006 according to the procedure described in section 3.1.4. Ten tins were coded for homogeneity tests, which should be performed by IVM.

3.1.3 QO06-4(Baltic herring)

Herring, originating from the Baltic Sea was purchased from Fishexport Hanstholm Frederikshavn - Chrisfish Danmark on 4 October 2006. After filleting (skin was not removed), the material was minced and stored at 0 °C. The material was homogenised and sterilised in tins on 5 October according to the procedure described in section 3.1.4. Ten tins were coded for homogeneity tests, which should be performed by IVM.

3.1.4 Details on the procedure for production of materials

The complete volume of meat was minced using a mincer (Finis Machinefabriek, Ulft) in combination with a Fryma mill equipped with toothed rotary knives (Fryma Maschinen AG, Rheinfelden, Switzerland) to a final size of 3.5 mm². Subsequently, ca. 25 kg sample (about 17 kg of QO06-3 material) was homogenised for 3 minutes, after adding 0.02% butylhydroxytoluene (BHT), in a Stephan cutter (Stephan Machines, Almelo, The Netherlands), type UMM/SK25 (made in 1979). For the QO-coded materials, coated tins (Eurocan Food, Mechelen, Belgium, volume ca. 75 ml) were filled to the brim with homogenised material using a manual dosing machine (maschinenfabrik Engler, Vienna, Switzerland). The tins were sealed by a Lanico TVM 335 sealing machine (Thomassen and Drijver, Deventer, The Netherlands). For the QM-coded material, glass jars were filled with ca 50 g of material and closed with the lid. The tins and jars were sterilised in a Muvero-Mat sterilizer (type 90E) for 45 minutes at 122 °C (pressure 1.4 bar, heating-time: 90 minutes, cooling time: 20 minutes).

3.2. Homogeneity test

Homogeneity test was performed for QM06-1 material by duplicate analysis of mercury in 10 glass jars (ISO-Guide 35, 2001). The determination of the total mercury concentration (the anorganic and organic fraction) was carried out according to ISW A021. The samples were microwave destructed with 10% nitric acid and subsequently the total mercury concentration was determined by flow injection analysis and flameless atomic absorption spectrometry. The determination is accredited under ISO17025 lab no. L097 and the quality is assured by internal and external reference materials, blank and recovery tests and by the participation in interlaboratory studies.

For evaluation of the homogeneity, the Soft CRM software of BCR is used, which comprises ANOVA statistics.

4. Results and discussion

4.1 Homogeneity test

The homogeneity results for material QM06-1 are shown in Table 2. The graph of the distribution of the means of the duplicate analysis is shown in Appendix 1.

Table 2. Homogeneity results of material QM06-1 for Hg (mg/kg ww).

| Sample ID | Analysis #1 | Analysis #2 | Mean | STDev |
|-----------|-------------|-------------|--------|--------|
| 10 | 0.0112 | 0.0114 | 0.0113 | 0.0001 |
| 40 | 0.0111 | 0.0113 | 0.0112 | 0.0001 |
| 70 | 0.0113 | 0.0106 | 0.0110 | 0.0005 |
| 100 | 0.0113 | 0.0111 | 0.0112 | 0.0001 |
| 130 | 0.0109 | 0.0122 | 0.0116 | 0.0009 |
| 160 | 0.0114 | 0.0115 | 0.0115 | 0.0001 |
| 190 | 0.0114 | 0.0111 | 0.0113 | 0.0002 |
| 220 | 0.0111 | 0.0109 | 0.0110 | 0.0001 |
| 250 | 0.0114 | 0.0109 | 0.0112 | 0.0004 |
| 280 | 0.0117 | 0.0108 | 0.0113 | 0.0006 |
| | | Mean | 0.0112 | |
| | | STDev | 0.0002 | |
| | | CV(%) | 1.6149 | |
| | | n | 20 | |

ANOVA Table

| Source of Variation | SS | d.f. | MS | StDev | F | F-crit 95% | F-crit 99% |
|---------------------|--------|------|--------|---------|-------|------------|------------|
| Between Units | 0.0000 | 9 | 0.0000 | MSB<MSW | 0.376 | 3.020 | 4.942 |
| Within Units | 0.0000 | 10 | 0.0000 | 0.0004 | | | |
| Total | 0.0000 | 19 | | | | | |

Snedecor F-Test

Differences between units statistically significant? (a=95%) :No

Differences between units statistically significant? (a=99%) :No

As is shown by the F-test, the material can be considered homogeneous, both within and between jars. The CV 1.6% is very low and will not contribute significantly to the between lab CVs in an ILS.

5. Conclusions

Three biological reference materials, 300 glass jars of homogenized Mediterranean mussels (QM06-1), 280 tins of homogenized blue mussels from German Bight (Q006-3) and 300 tins of homogenized Baltic herring fillets (Q006-4) were successfully prepared.

The homogeneity was tested for QM06-1 material by duplicate analysis of mercury concentration in 10 glass jars and subsequent statistical evaluation of the results. The between- and within homogeneity found to be satisfactory. The variances as observed in these homogeneity tests were all much lower compared to values normally observed in interlaboratory studies. The material is suitable for a trace metal contaminant interlaboratory study.

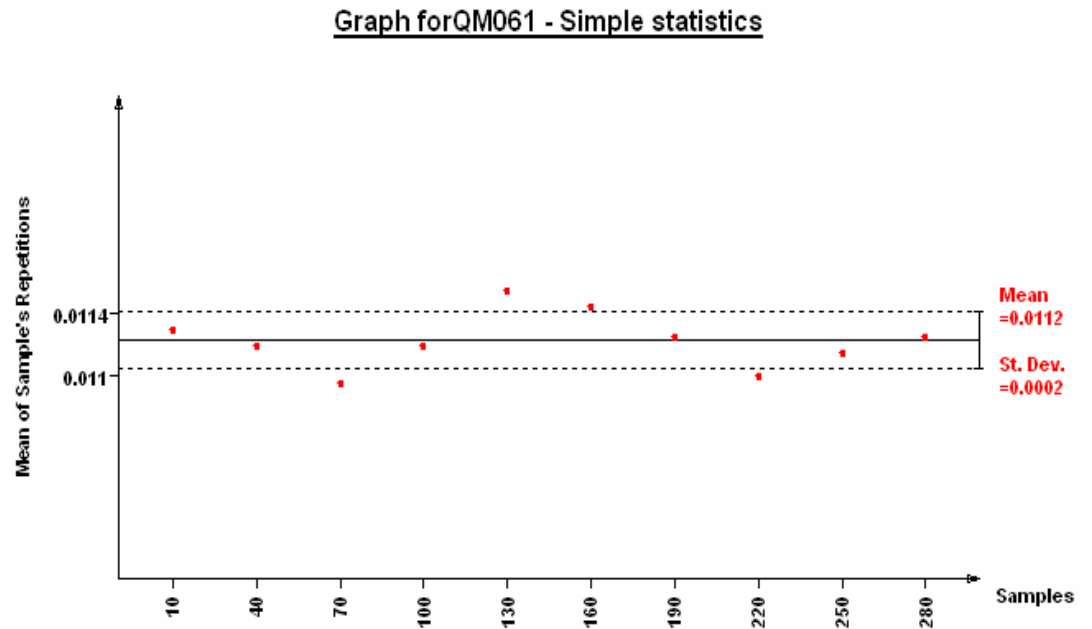
The other two biological materials (Q006-3 and Q006-4) were not tested for homogeneity.

6. References

- ISO guide 35 (2001). Certification of reference materials - general and statistical principles, 3rd ed., draft 2. Geneva, International Organization for Standardization.
- QUASIMEME (2003). QUASIMEME laboratory performance studies, BT-1 Trace metals in biota, round 32 - exercise 549, QUASIMEME, Aberdeen.

Appendix 1. Homogeneity graph (distribution of the means of duplicate analysis)

Figure 1. Means of two replicate analysis of each jar in the homogeneity study for Hg in material QM06-1



Signature: _____

Date: 7 December 2006