ASSESSMENT OF HOMOGENEITY

What is Homogeneity?

Homogeneity can refer either to variation of a property value between separate units of the material, or to variation within each unit. It is always necessary to assess the between-unit variation.

Where the intended use permits the use of part of a unit – for example, a small portion of a solid or liquid material, or a small region of the surface – it is also usually necessary

- ✓ either to assess the within-unit variability of the material (within-unit heterogeneity) or
- ✓ to provide instructions for use that control the impact of within-unit heterogeneity.

These instructions can include, for example, remixing of the sample and, for granular materials, a minimum sample size, because the within-unit heterogeneity is directly reflected in the minimum size of subsample that is representative for the whole unit.

When homogeneity assessment is required?

Materials of natural origin or with complex matrices, such as foodstuffs, soils, ores and alloys, are typically heterogeneous in composition.

RMs prepared as pure compounds or solutions of pure compounds are expected to have a high degree of homogeneity. These materials can, however, also show some heterogeneity, for example, due to a density gradient, localized contamination, evaporation of solvent during processing or filling, variations in residual solvent content, or metals containing variable amounts of occluded gases.

Components of Homogeneity

Homogeneity is an important requirement for all RMs and includes **both within- and between-unit homogeneity**.

Between-unit homogeneity is important to ensure that each RM unit carries the same value for each property;

Within-unit homogeneity is important where subsamples can be taken for measurement by users of the material.

Homogeneity assessment

The results of an experimental homogeneity study are usually also used for the calculation of one of the uncertainty components in the certification model .

Based on current practice, an acceptable estimate of the between-unit variance for the purposes of uncertainty evaluation can be obtained with nine or more degrees of freedom. For a simple, homogeneity study, this corresponds to the selection of a minimum of 10 units.

As the total number of units produced, N_{prod}, increases the number of of units selected should be increased. Recommended no. is given as

No. of units for Homogeneity study = Maximum of $(10, N_{prod}^{1/3})$

It is not normally useful to examine more than 30 units of a reference material characterized for a quantitative property.

If an RMP prepares 3 000 units of a candidate RM and intends to undertake a homogeneity study in a single run, the number of units to be taken for homogeneity comes to 14.4. i.e 15

Homogeneity assessment

Where the batch size is below 100 units, homogeneity should be assessed on the larger of 3 units or 10 % of the batch size, randomly selected from the batch.

Where homogeneity is to be determined experimentally it is usually necessary to determine the homogeneity for every property of interest;

To obtain reliable results, it is important to

— choose the properties to be studied,

— select a representative subset of units,

— choose a suitable measurement procedure with sufficient repeatability and selectivity,

— make the measurements under suitable conditions following an appropriate experimental design, and

— conduct the statistical analysis using valid statistical methods.

SYMBOLS USED IN RMP STATISTICS

y_{char} – Certified property value
S_{bb} – Between unit SD
S_w - Within unit SD
S_r – Repeatability SD and S_R – Reproducibility SD
u_{char} – Standard uncertainty due to characterization
u_{bb} – Between unit (standard) uncertainty
u_{wb} – within unit (standard) uncertainty
u_{hom} - Homogeneity (standard) uncertainty
u_{lts} – Uncertainty (standard) due to long term stability (storage) -
u_{sts}- Uncertainty (standard) due to short term stability (transport)
u_{trg}- Target standard uncertainty
u_{CRM} – Standard uncertainty of the certified Value
U_{CRM}- Expanded uncertainty of the certified Value

SYMBOLS USED IN RMP STATISTICS

u_{CRM} = Sq. Root of ($u_{char}^2 + u_{hom}^2 + u_{lts}^2 + u_{sts}^2$)

u_{hom} = Sq. Root of ($u_{bb}^2 + u_{wb}^2$)

$U_{CRM} = u_{CRM}$ x coverage factor(k)

Where
u_{CRM} – Standard uncertainty of the certified Value
U_{CRM}- Expanded uncertainty of the certified Value
u_{char} – Standard uncertainty due to characterization
u_{bb} – Between unit (standard) uncertainty
u_{wb} – within unit (standard) uncertainty
u_{hom} - Homogeneity (standard) uncertainty
u_{lts} – Uncertainty (standard) due to long term stability (storage) -
u_{sts}- Uncertainty (standard) due to short term stability (transport)

Homogeneity assessment - Layout

Layout of Between unit homogeneity study (Fig. 2 in page 15 of ISO Guide 35). Use this when sub-sampling of RM is possible. – Between unit variance will be reasonable

Alternative layout of Between unit homogeneity study (Fig. 3 in page 16 of ISO Guide 35). Use this when sub-sampling of RM is not possible - Between unit variance will be over estimated.

Homogeneity Study designs

3 designs considered are illustrated in **Figure 4. (Page 17 of ISO Guide 35).**

a) Simple randomized design : Single run with all units observed in duplicate in random order. All observations may be acquired in a single measurement run.

Calculate between unit uncertainty contribution,

$u^2_{bb} = [MS_{between} - MS_{within}] / No.\ of\ replicates$

USE ONE-WAY ANOVA (Example given in Annex C1)

EXERCISE - 1

Homogeneity Study designs

b) Randomized block design : Each run includes one observation for every unit and each run being separately randomized.

This design is appropriate when the requisite no. of replicates on all units can't be included in a single run due to time constraints or instrument constraints

Here S^2_w (MS_{Error}) includes variability due to (a) Measurement procedure plus (b) due to within unit heterogeneity

Calculate between unit uncertainty contribution,

$u^2_{bb} = [MS_A - MS_{Error}] / No.\ of\ replicates$

USE TWO-WAY ANOVA WITHOUT REPLICATION

(Example given in Annex C3) – EXERCISE -2

Homogeneity Study designs

C) Nested design : (e.g) 4 units are randomly allotted to each of the three runs and duplicate observations are made on each (in a randomized order)

Here the degrees of freedom associated with between unit variation will be reduced. Hence use more no. of RM Units for the study.

This design is appropriate in case of collaborative study (ILC) used for both (a) homogeneity assessment and (b) characterization of RM.

Calculate between unit uncertainty contribution,

USE TWO-WAY ANOVA WITH REPLICATION

(Example given in Annex B2)

WITHIN UNIT HOMOGENEITY

Situations involving a high risk of **appreciable within-unit heterogeneity** include one or more of the following:

— minimum sample size substantially less than the unit size; (RM unit is used repeatedly)

— a material prepared by mixing powders or granular materials; **(Minerals, Soil)**

— a material prepared by mixing a small quantity of one component into a bulk matrix; or

— a material with previously known within-unit heterogeneity.

Experimental assessment may consist of

- ✓ **a test for significant within-unit heterogeneity** or
- ✓ **a determination of minimum sample size**.

WITHIN UNIT HOMOGENEITY

Test for significant within-unit heterogeneity

Experimental design is similar to between-unit homogeneity studies, with the exception that the variance of interest is the within-unit, between-subsample term

- ✓ **Choose layout in Figure 5(a) (Page 24 of ISO Guide 35) when sufficient no. of subsamples can be taken from one RM unit. Figure 4.**
- ✓ **Otherwise choose layout in Figure 5(b) (Page 24 of ISO Guide 35)**

WITHIN UNIT HOMOGENEITY

Test for significant within-unit heterogeneity

A test for within-unit heterogeneity should provide at least 5 degrees of freedom for the within-unit term.

This can be achieved by:

- ✓ **1 RM unit and 6 or more sub samples from this unit**
- ✓ **3 or more RM units and 3 or more sub samples from each unit**
- ✓ **5 or more RM units and 2 sub samples from each unit**

WITHIN UNIT HOMOGENEITY

Layout in Figure 5(a) is chosen

Use this approach only when 6 or more subsamples can be taken from one RM unit

Estimate within unit SD, S_{wb} using one way ANOVA

Use subsample as the grouping factor

EXERCISE - 3

WITHIN UNIT HOMOGENEITY

Layout in Figure 5(b) is chosen

Use this approach only when sufficient subsamples can't be taken from one RM unit

Estimate within unit SD, S_{wb} and corresponding standard uncertainty u_{wb} using Two way ANOVA with replicates

Estimate between unit SD, S_{bb} and corresponding standard uncertainty u_{bb} also in this case

EXERCISE - 4

Determination of minimum sample size

Carry out a within-unit homogeneity study for different sizes of sample intake. Normally as the sample intake reduces the observed within-unit homogeneity SD will increase

- ✓ Determine within unit SD, S_w for a range of different sample intake (say 10 mg to 50 mg of RM)
- ✓ Plot a graph between observed S_w against sample intake – SHOW EXCEL
- ✓ Either demonstrate that sample intake has no effect on S_w , or
- ✓ Choose a sample intake at which estimated S_w is nearer to repeatability SD, S_r of the measurement procedure (for granular materials – ores, soil etc.,)

Check for "sufficient" homogeneity

RM producer should confirm that the variation within and between units S_{wb} / S_{bb} is **sufficiently small for the intended use of the material**.

- ✓ **Comparison** of S_{wb} / S_{bb} to the **uncertainty associated with characterization** to confirm that the standard deviation(s) are small compared with the characterization uncertainty (for example, $S_{bb} < u_{char}/3$);
- ✓ **Combined uncertainty u_{CRM} of the certified value** (which includes S_{wb} & S_{bb}) **is acceptable for the intended use**
- ✓ **When u_{CRM} is not calculated for the RM, check that $S_{bb} < S_R/3$. where S_R is the interlaboratory reproducibility standard deviation**
- ✓ use of an F test, that the **between-unit term** is not statistically significant at the 95 % level of confidence.

Uncertainty evaluation from homogeneity studies

- ✓ **Where there is significant within-unit heterogeneity, u_{wb} at the minimum sample size to be recommended in the instructions for use, the uncertainty associated with a certified value should be increased by the inclusion of a further allowance u_{wb}**

  u_{hom} = Square root of ($u_{bb}^2 + u_{wb}^2$)

- ✓ **The contribution u_{hom} to the uncertainty of a certified value may also be increased to allow for insufficient precision of the measurement procedure used for homogeneity studies, as provided for in example C2**