



## INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)

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### OVEN SEED MOISTURE TESTING. 0. VALIDATION COMPARATIVE TESTING

#### (ISTA MOISTURE COMMITTEE 2005-2009)

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### 1. Introduction

#### 1.1. General

The objective of this comparative testing was to generate guidelines that would make it possible to validate the introduction of new species for the moisture chapter of the ISTA Rules either by

- using the constant temperature oven method instead of a standard reference method;
- by using the oil content level as separator between methods, without the necessity to do comparative validation testing for each new species; and
- at the same time to be able to change the method for species currently wrongly placed in the ISTA Rules.

Factors that have an influence on the determination of Moisture Content are:

1. seed constituents (particularly volatile components)
2. particle size (where seed is ground as part of the determination)
3. seed coat permeability.

Factor 1. is dealt with in this comparative testing. Factors 2. and 3. will be dealt with in separate studies. These will involve literature review and consultation and discussion with experts within ISTA and from other organisations such as ISO, ICC, etc.

Ten species for which grinding is not necessary were chosen in order to avoid grinding being a factor in the test results. In addition, no legumes or clovers were included to avoid impermeable seed coats being a factor.

A group of 13 laboratories performed the tests according to the defined procedure on a set of samples. Participating laboratories were either accredited for moisture testing by ISTA, or had attended the ISTA Moisture Workshop in Copenhagen in 2003.

#### 1.2. Statistics

The statisticians of the ISTA Statistics Committee carried out simulations, based on true values of moisture tests and expected differences between two replicates and two methods supplied by chairperson of the Moisture Committee and on data from the last proficiency test supplied by the ISTA Secretariat.

From these two sets of information the ISTA Statistics Committee (STAT) generated different sets of raw data "as could be expected" with means and variability. These data were generated for different numbers of laboratories. After statistical analysis this gave an indication about figures that could be expected from the comparative testing.

The main messages from these computations were:

- One lab should prepare the samples for the different modalities at the same time, and select at random which samples are used for which method.
- Not to restrict the number of labs to the minimum of six labs, but increase it to 10-15 if possible. (Generally method validation will involve six laboratories testing only one method (ISTA Validation Handbook). For this moisture comparative testing the situation was different, as there are two methods (103°C and 130°C) and two factors (oil content and moisture content.) Based on statistical estimations made by the ISTA Statistics Committee a minimum of 10 laboratories is necessary in order to obtain useful data from the trial that could be used for validation purposes. The optimal number of laboratories



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would be 20. With 15-20 laboratories participating laboratories it would be possible to see a general tendency across the levels, and by level of moisture. It would also make it possible to discard outlier results or laboratories (up to four).

- If the difference between two means is about 0.05% (compare two methods at a given level) (compare successive oil levels for a given method) the analysis will probably conclude that there is no difference.
- If the difference between two means is about 0.1% (compare two methods at a given level) (compare successive oil levels for a given method) the analysis will probably conclude that there is a difference.

Based on statistical estimations, it was decided to include eight species in this trial. The species were chosen to represent the full range of oil content found in seeds. Each species was conditioned to two moisture levels and the samples of each species (8) at each moisture level (2) were tested by 10-15 laboratories.

The statistical analysis is based on the assumption of one value per sample.

For every sample received, each participating laboratory drew six sub-samples for moisture content testing. At random two of these were used for moisture determination at 130°C (time study); two for moisture determination at 103°C (17 hrs only); and two for moisture determination at 103°C (time study). As even 103°C may be too high in case of high oil contents, 70°C was also considered an option. However, this temperature was discarded for the following reasons:

- Other international organisations don't have this temperature in their Rules.
- Not all water will evaporate within one week.
- It would make the comparative testing too complicated and too laborious for the participating laboratories.

From a given lot sent to the laboratories, the laboratories obtained different results depending on the true value of the sample they received and the accuracy of their testing. For this reason it was necessary that the laboratories drew six sub-samples from a received sample and used two for each method, instead of having three separate samples, one for each method.

The advantage of having two sub-samples for each test at a particular temperature is that a value for the variability within the laboratory is obtained. If only one sample for each determination was used and the laboratories obtained different results, it is not possible to determine whether the differences are due to the method, or due to the variability within individual participating laboratories.

In the end it was decided to send 20 samples to 13 different laboratories.

## 2. Validation of data

### 2.1. Equilibration (moisture of samples) and dispatch

The official seed-testing laboratory for Scotland (SASA) was responsible for the conditioning of sample moisture content and dispatch of samples to participants. Once all the samples (lots) were received, each lot was divided into two and the two halves were placed in controlled RH rooms one at ca 30% RH and one at 80% RH. The seed was kept in these rooms for some days to allow the conditioning of moisture content (MC). The conditioning



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period was too short to be sure equilibration of the seed samples with their environment and the laboratory therefore performed a heterogeneity tests on the moisture contents of the sample (10 species conditioned at 2 RHs resulting in 20 samples each of which had a heterogeneity test carried out on it)..

See table 2.1 for the species that were used.

After conditioning species were removed individually (from the high and low humidity rooms) and each was divided into at least 30 samples using the procedures as described by Don (2003). From each original seed lot of a species 60 sealed foil packets - 30 at a low and 30 at a high moisture content - were obtained. Each of the 60 samples from each species had a weight of at least 30g. Ten foil packets for each species and for each moisture content level were tested for heterogeneity. The temperatures used for this moisture content test were: *Lolium*, *Poa*, *Lycopersicum*, *Petroselinum* at 130°C (for 1 hour); all other species 103°C (for 17 hours).

The results of the heterogeneity moisture contents were sent to the ISTA Proficiency Committee for analysis. The results indicated that all samples were homogeneous (table 2.1.).

**Table 2.1.:**

Heterogeneity of moisture content, based on ten samples per species.

	<i>Lolium perenne</i>		<i>Spinacia oleraea</i>		<i>Poa pratensis</i>		<i>Lycopersicum esculentum</i>		<i>Allium cepa</i>	
	1	2	3	4	5	6	7	8	9	10
Sample Mean	5,70	24,00	5,90	20,50	6,50	16,70	5,00	14,40	6,00	17,80
Range between tests	0,10	0,30	0,10	0,30	0,00	0,10	0,10	0,10	0,00	0,10
Sample Homogeneity	OK	OK	OK	OK	OK	OK	OK	OK	OK	OK

	<i>Petroselinum crispum</i>		<i>Sinapis alba</i>		<i>Camelina sativa</i>		<i>Brassica napus</i>		<i>Linum usitatissimum</i>	
	11	12	13	14	15	16	17	18	19	20
Sample Mean	5,20	15,20	4,90	15,20	4,30	18,10	3,50	17,70	4,30	11,80
Range between tests	0,20	0,10	0,10	0,20	0,10	0,10	0,10	0,10	0,20	0,00
Sample Homogeneity	OK	OK	OK	OK	OK	OK	OK	OK	OK	OK

Finally the validation comparative study had the following lay out:

- 10 species covering a large range of oil contents.
- 2 moisture levels, the samples were conditioned to low and high moisture level.
- 2 methods high and low temperature (1 hour 130°C and 103°C for 17 hours )
- A time study, where the working samples were weighed after 1,2,3,4,5,6,7,8, 17, 24, 48 , 72 and 96 Hours in the oven.
- 2 replicates for each determination
- 13 laboratories.



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To ensure that the results used for the final statistical calculation were representative and validated, all the results were computed and a search for all results that seem to deviate from the norm was performed using a range of methods. . The outcome of this search is given below.

### 2.2 Results of enquiry

Participating laboratories were requested to indicate if they had to deviate from the supplied Standard Operating Procedure (SOP) for the comparative test. Two labs reported deviations and four labs were not able to report all the required information.

Lab 5 shifted 17hrs in the time study to 24 hrs. The graphs show no visible influence of this difference of 7 hours. Lab 4 indicated interrupted electricity on days 3 and 4 during the time study. It was for a limited period only, and no effects on results could be detected. Therefore the results were retained. Lab 10 did not carry out all tests. Results are missing for sample 1 for both temperature time studies. In addition, at 103°C three individual values are missing: sample 4b, one replicate after 72 hours, and sample 5a replicates at 48 and 72 hours. For these times, the average is based on the results of 9 labs only: from a statistical point of view this doesn't generate any problems,

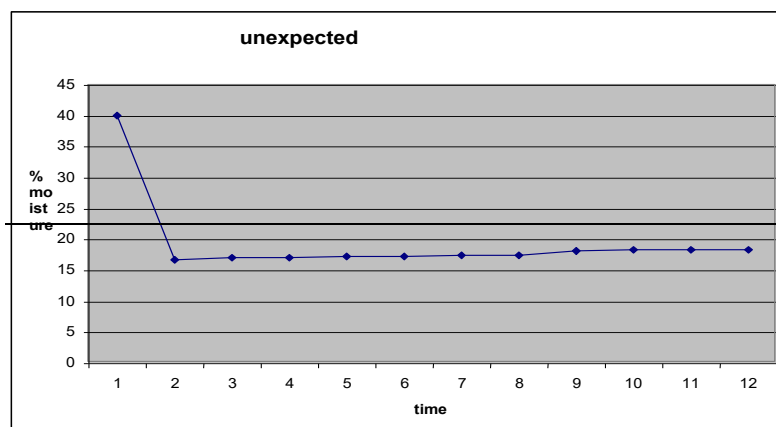
Three labs (lab codes: 3, 6, x) did not have information on the variation in temperature at different positions in the oven. However, as no information as to the effect this has is available from the labs that do know this variation, this cannot be taken into consideration when drawing the conclusions.

### 2.3. Check time studies for deviating results (deviating averages)

The next step was to identify deviating results by comparing the individual results per combination of species \* moisture level. Deviating labs were contacted, and corrections made if the deviation was due to a typing error (Figure 2.1), or results retained if no error was found (Figure 2.2).

**Figure 2.1:**

Deviating averages for Lab 4, sample 5b *Alium*





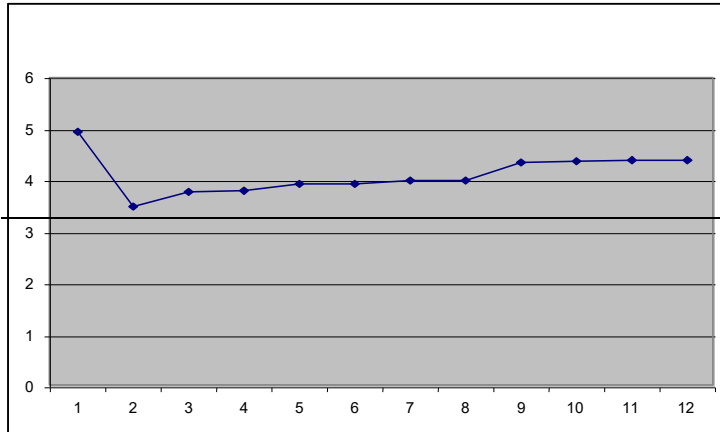
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### Figures 2.2:

Deviating averages for 4a *Linum* Lab 5 unexpected 4.93



For some of the questionable results, no reason could be found and they were probably the result of experimental variation. These results were kept, and used for calculations. As only a very limited number of questionable results were reported, the average of the 10 labs was hardly influenced by these.

### 2.4. Check time studies for deviating results (differences between replicates)

A GLM analysis of variance in Statgraphics was carried out, resulting in the standard deviation of replicates.

Sample

T°

lab

Sample\*T°

T°\*lab

T residuals were calculated. Values greater than 6 were judged to be outliers. This analysis resulted in five data points greater than 6, as represented in the following graphs (Figures 2.3). The conclusion is given in each graph.

### Figures 2.3:

Deviating replicates; for details of individual deviating replicates see the heading of the individual graphs. In each case:

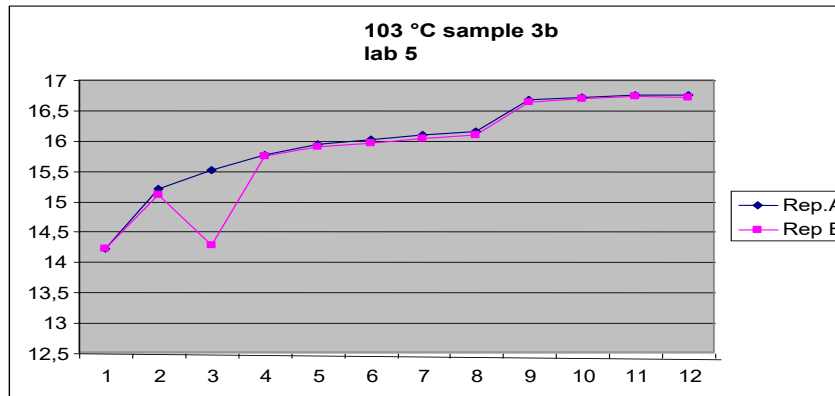
- x-axis is order of consecutive measurements in time study
- y-axis: moisture content



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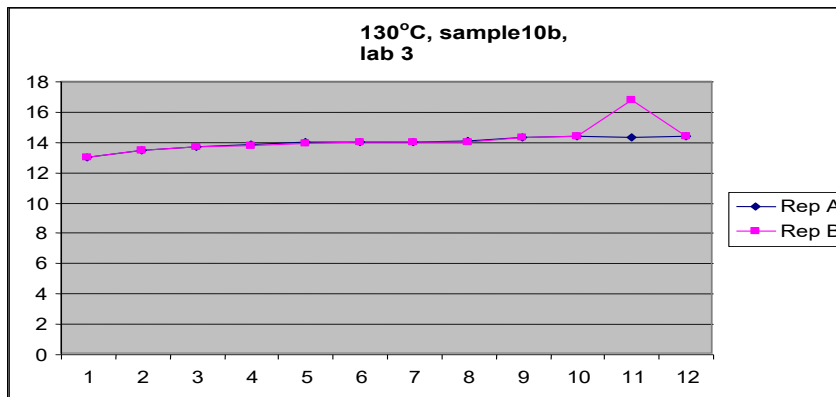
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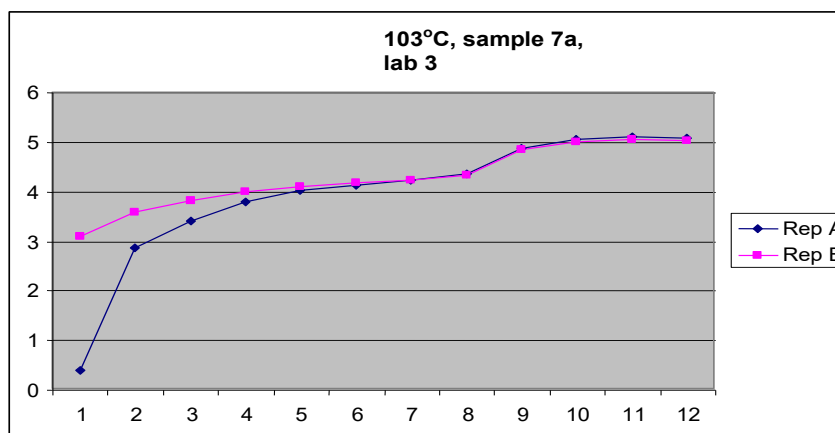


The result was checked, and no mistakes could be found.

As the result is obviously a mistake, it was discarded and treated as 'missing value'.



This deviating replicate was the result of a mistyping of the weighing result: 38.807 grams was reported, whereas 39.807 grams was measured. The result was changed accordingly in the table.



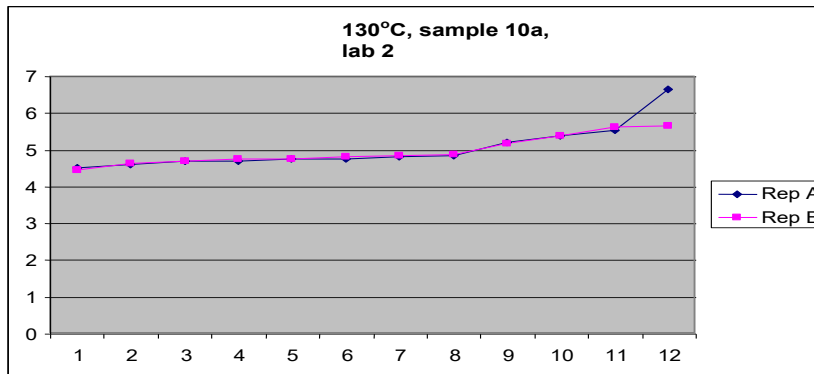
The lab checked all steps of the moisture testing, but didn't find an answer to this deviating result. As we cannot be sure which curve is the correct one, and as we are not sure if the two curves are really the same, the results were kept.



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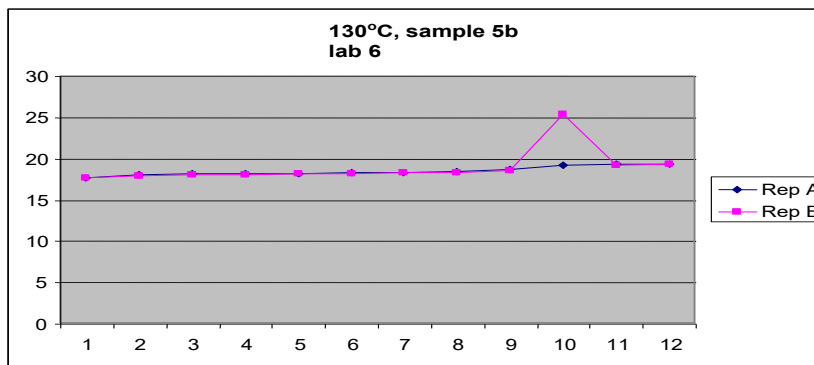
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There appeared to be no typing error, although it could not be excluded that a wrong figure has been written when reading the weight on the balance.

As the result is obviously a mistake, it was discarded and considered as 'missing value'.



After checking the lab reported that a mistake had occurred when copying the original hand written results to the supplied form. The result in the tables was corrected accordingly.

With residual analysis, three additional deviations were found, indicated by arrows in the following three figures (Figures 2.4, 2.5 and 2.6).

Results were considered different from the others if 8 to 11 residuals were >6 for the 12 data points of the repeat. The graphs that are provided here are less easy to read and less convincing than for the data points shown above. In two cases one repeat is lower than the others and in one case one repeat is higher..

### Figures 2.4:

Results of 103°C, *Lolium*, sample 7a.

In one lab (indicated by the arrow) one of the two subsamples is 8 out of 12 lower than all others labs.

- x-axis: lab (two results per lab),
- y-axis: moisture content,
- legend: series=result of consecutive readings in time study

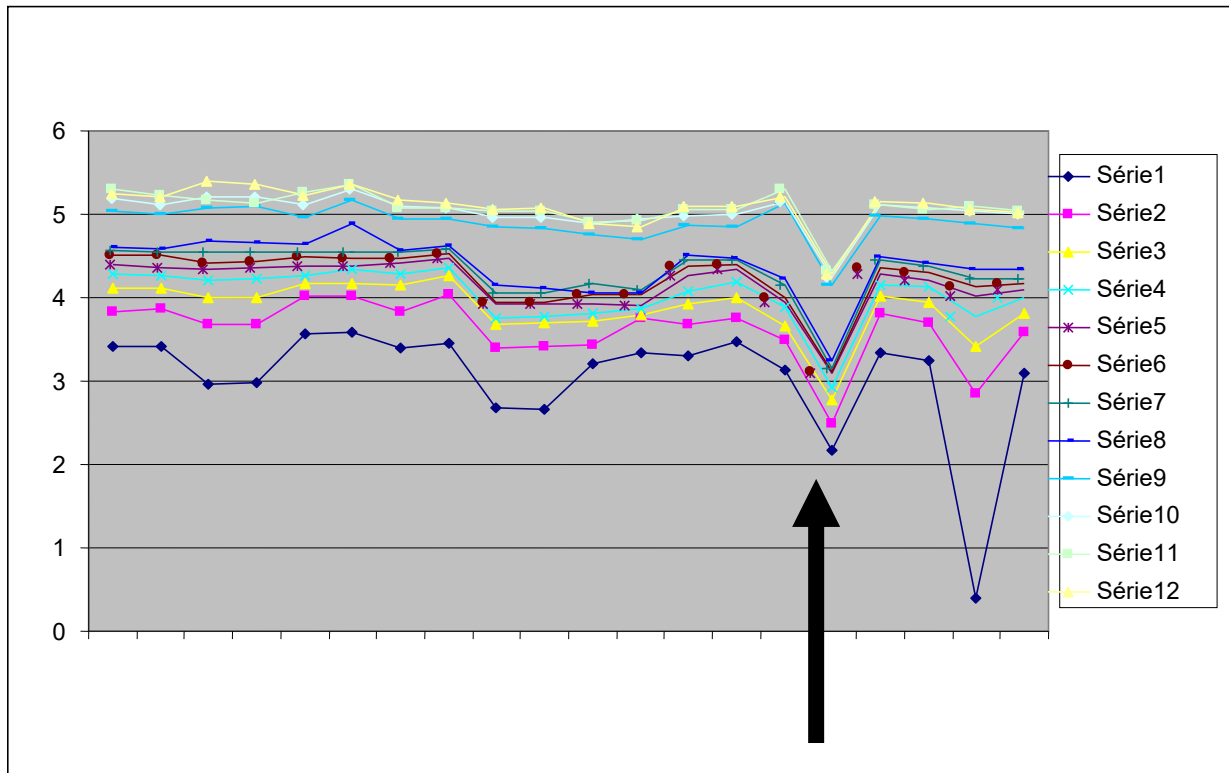




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In this case (Figure 2.4) a weight of 29.013 g was reported. It was checked, and a weight of 29.056 g was measured. The figure was replaced accordingly in the tables.

### Figure 2.5:

Results of 103°C, *Lolium*, sample 7a.

In one lab (indicated by the arrow) one of the two subsamples is in 9 out of 12 lower than all others labs.

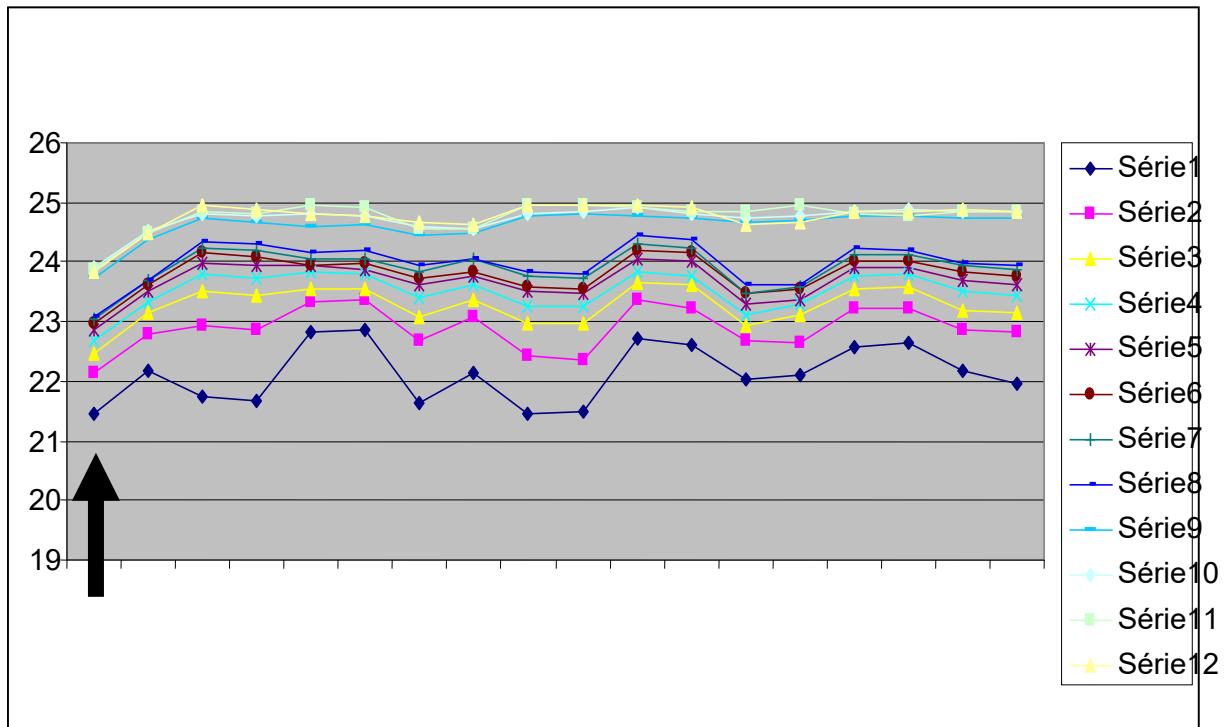
- x-axis: lab (two results per lab),
- y-axis: moisture content,
- legend: series=result of consecutive readings in time study



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There appeared to be no typing error, although it could not be excluded that a wrong figure has been written when reading the weight on the balance. It was decided to keep the results (Figure 2.5).

### Figure 2.6:

Results of 130°C, sample 9a, lab 5.

In one lab (indicated by the arrow) one of the two subsamples is in 11 out of 12 higher than all others labs.

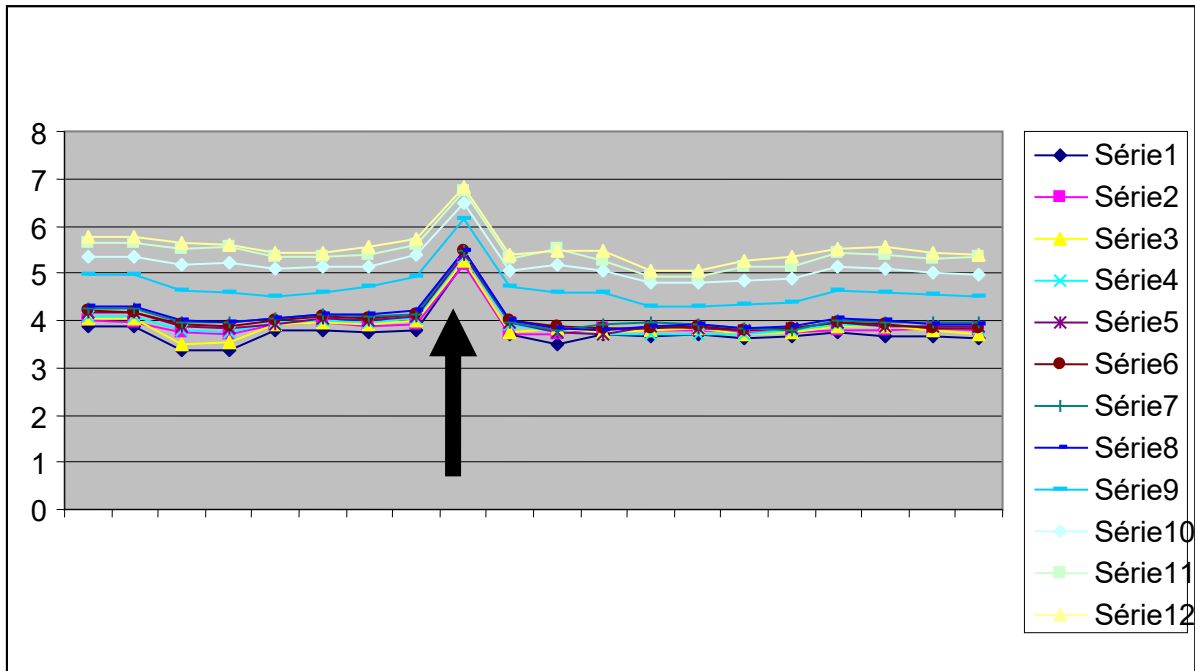
- x-axis: lab (two results per lab),
- y-axis: moisture content,
- legend: series=result of consecutive readings in time study



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This lab (Figure 2.6) indicated that it had observed the deviating results during the experiments. It could not find any reasons for this however. It was decided to keep the results.

### 2.5. Check for deviating results by means of Z-score analysis

The major part of this research consisted of two time studies. Later weighings should result in increasing moisture losses (until stable), resulting in lower weights being reported. Occasionally an increase of weight was reported. If increases in weight regularly occurred, results were rejected, suppressed in the tables, and considered as being 'missing' in the statistical calculations.

Z-scores were calculated; see Annex 1 for results. A z-Score is the difference between the value observed by a lab and the mean of all observed values for the same sample, divided by the variability of all labs on this sample (standard deviation of the results of all labs on this sample).

A negative value indicates that the result is below the average of the labs.

A positive value indicates that the result is above the average of the labs.

95% of the z-scores should lie in the interval  $[-2,+2]$  if all labs "perform similarly".

In Annex 1 the values in this interval are green ( $<0$ ) and blue ( $>0$ ) when they are in the interval  $[-2,+2]$ . The values left blank are outwith the interval  $[-2,+2]$ .

With 280 data points (14 labs\*20 samples) 5% , i.e.14 data points, are expected to be outside the interval  $[-2,+2]$ . In this dataset are 21 z-scores were outside the interval, which is more than expected. Lab Y had 13 "out of interval" z-scores, all being positive. This is an indication that Lab Y has results that are above the average of all labs.

#### Z-score

If the sums of the 20 absolute z-scores for each lab are computed, this sum can be compared with the expected sums of z-scores for 20 values. The expected sums are:

95% of the time this sum is less than 20.6



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99% of the time this sum is less than 22.7

99,99% of the time this sum is less than 27

This confirms that Lab Y has results that are above the average of all labs (sum of z-scores = 41,3).

Looking at lab Z all the z-scores are negative (= always underestimate compared to average of all labs) and the sum of absolute z-scores is 21,9 (between 95% and 99% quantiles) All other labs are below the 20.6 value for the sum of 20 z-scores

Lab 10 has only 18 data points for which the:

95% quantile is 18.7

99% quantile is 20.7

The sum is 19.9 so we have a similar situation to lab Z (all the z-scores negative and the sum of absolute z-scores is 21,9 (between 95% and 99% quantiles).

To summarise this analysis of z-scores from the table:

1. Lab Y is very significantly over estimating compared to the average of all labs.
2. Lab Z and Lab 10 are under estimating compared to the average of all labs.
3. For the other labs, the analysis of z-scores does not highlight a lab as any of these as being different from the average of all labs

The results with z-scores greater than +/- 2 were not excluded from further analysis.

The results outside the interval are a type of alert. However, if there is no reason to doubt the correctness of the results they should be retained (see further section 2.6.).

There are also instances where mistakes may have been made, e.g. the unexpected large loss of weight (resulting in large increase in moisture content) between two periods in the time trial of some of the labs. Lab Y's results could be due to the loss of seed rather than a change in moisture content.

### 2.6. 'Crossing Lines' in the time studies

The 40 graphs (10 species \* 2 moisture levels \* 2 oven temperatures) were checked by looking at 'crossing lines'. Crossing lines indicate increasing moisture contents over time. This is an indication that a result is not correct, as in principle moisture is lost in the oven. See Annex 3 for an example of such an evaluation.

The reason for these observations may have been:

- Moisture attraction during cooling down
- Temperature of the oven not correct
- Oven not being closed
- Incorrect weighing.
- Incorrect calibrations.

By checking the graphs three groups of deviating results were found:

#### Deviating results from Lab Y

Lab Y had many deviating results in the time studies. A number of times the value after one hour is higher compared to the later ones; the ones after 72 and 96 appear to be ok again. Loss of seed would mean that all values should be high. Other possible reasons for the deviations include:

- Attraction of moisture during cooling down (wet conditions during part of experiment?).



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- Balance not operating properly.
- Weighed wrong samples, or noted results in wrong place.
- Oven temperature wrong during part of the experiment.

### Deviating results from Lab Z:

All 130-high-moisture results from Lab Z are very low; it looks as if they had problems with the temperature of the oven. All 130-low moisture results from Lab Z are relatively low; these results may (partly) have z-scores smaller than +/-2. Even if this would be the case they don't seem to be right. At 103°C the results after 1 hour are very low; later results seem to be ok, except for the *Linum* high moisture content sample.

### Deviating results from Lab X:

There is a problem with the 4 hours at 130°C results of Lab X for five species (*Lolium*, *Spinacea*, *Poa*, *Lycopersicum*, *Petroselinum*). These data-points have a higher moisture contents than later readings. In addition, there seem to be problems with the results of: *Sinapis* samples tested at 130°C after 6 hours; *Camelina* samples tested at 130°C after 7, 8 and 96 hours; *Brassica* high moisture sample tested at 130°C after 2 and 96 hours; *Brassica* low moisture sample tested at 130°C after 6 hours; and *Linum* low moisture sample tested at 130°C after 2 hours.

The results from Lab Y, Lab Z and Lab X were considered unreliable: they all reported multiple 'crossing lines', thus demonstrating obvious mistakes. The results of these labs were therefore all discarded in all further calculations.

Of these labs, lab Y reported excessively long recovery times of the ovens they used ( 65-120 minutes) and it did not calibrate its temperature measuring equipment. This may have been the cause for the deviating results. Labs X and Z showed no deviating aspects in their answers to the questions regarding testing procedures or equipment.

Apart from the problems mentioned above, only occasional outliers and/or crossing of lines were observed. In a few cases the first reading was sometimes low, e.g. lab 3 for the low moisture *Sinapis* sample tested at 103 °C, and some lab-5 results when samples were tested at 103°C.

After discarding the results from Lab Y, Lab Z and Lab X the results from 9 labs for *Lolium* and the results from 10 labs for the other species were used for further statistical calculations.

## **2.7. Check standard deviations**

There are different ways to look for exotic data points. One very simple one is to compute the standard deviation. This was done for all samples and all labs, only for the data set 103°C for 17 hours.

The table of data on page 1 of Annex 2 shows these standard deviations and a graph of them. The bigger the value, the more different are the two repeats for the same sample in the lab. In the table, values above 0.5 are indicated in orange, values above 1.0 in red. The values above 1.0 are analyzed in further detail in the graphs following on pages 2, 3 and 4 of Annex 2. The results of labs X, Y and Z, which were discarded, are included in italics (grey), and were not taken into consideration for these calculations.



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On page 2, 3 and 4 of Annex 2 standard deviations are p; the instances where the standard deviation is the greatest are plotted. All results were reviewed "one by one" from the graphs of all results for the sample where a problem was noticed. There is no general norm for discarding results based on deviating results and decisions are taken based on what is observed in the graphs.

The graph on page 2 of Annex 2 shows the results for *Lycopersicon* – high moisture content sample. One replicate of lab 7 is totally out of range and the result of this replicate was discarded. The results on page 3 of Annex 2 (*Lycopersicon* – low moisture content sample) and page 4 of Annex 2 (*Poa* – low moisture content sample) indicated deviating results, but in these cases the results are still of the same order as the other results. For these two cases the difference between the two repeats is large (more than 1%) but looking at all data for the sample over all labs, the deviating values do not appear to be as big as in the case of the *Lycopersicon* – high moisture content sample. For this reason the deviating results were not discarded.

For repeatability computations at least two values per lab are needed on a sample. This means that by discarding one repeat, we have only one data point left. The discarded result of *Lycopersicon*-high moisture content sample will therefore be suppressed from repeatability computations.

### **2.8. Statistical check of Karl Fischer results**

There was only one set of data for the Karl Fischer test and statistical analysis of the results did not reveal any problems.

### **2.9. Oil contents**

Samples from several sources were checked for oil content and statistical analysis of the results did not reveal any problems.

### **2.10. Conclusion on validation of data**

The results in the workbook were amended according to the above data validation.

Thereafter the workbook was used for making the calculations which were used for the publications listed in '5. References' (Nijenstein *et al*, 2009a, 2009b, 2009c)



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### 3. Repeatability and reproducibility of results

Repeatability and reproducibility calculations were carried out after the validation process (see 2).

#### 3.1. Repeatability (absolute difference between replicates)

Repeatability was first estimated by the median of the absolute difference between replicates, averaging all laboratories. The average for all differences is approximately 0.05% (see table 3.1.), and is slightly lower at 103°C than at 130°C. 95.5% of the values are smaller than 0.2% (table 3.2. and figure 3.1.).

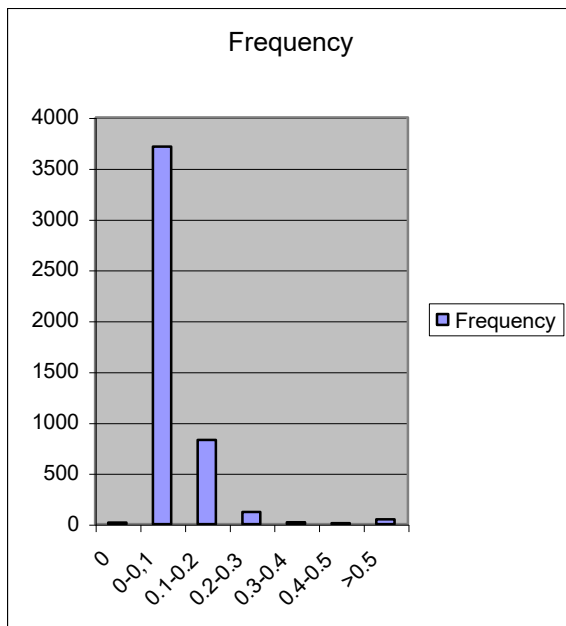
**Table 3.1.:**

Medians of the differences between two replicates of one sample.

103	0,054	0,049	0,050	0,044	0,044	0,043	0,049	0,042	0,033	0,035	0,033	0,033	0,044
130	0,058	0,047	0,046	0,056	0,047	0,047	0,044	0,052	0,053	0,051	0,056	0,053	0,052
avg	0,057	0,049	0,048	0,050	0,046	0,046	0,047	0,050	0,044	0,043	0,044	0,042	0,046
hours	1	2	3	4	5	6	7	8	17	48	72	96	avg

**Figure 3.1.:**

Frequency distribution of absolute differences.



Class	Frequency	%
0	17	0,4
0-0,1	3713	78,1
0.1-0.2	829	17,4
0.2-0.3	119	2,5
0.3-0.4	19	0,4
0.4-0.5	9	0,2
>0.5	46	1,0

The variability of the results within the labs was calculated by computing the variances between the two replicates for each lab-sample-method. The width of the bar is computed by Analysis of Variance. The width is the same for all labs in these graphs, as analysis of variance computes an average variability. If two bars have no overlap, analysis of variance would conclude to a significant difference between the two laboratories (fig 3.2.)



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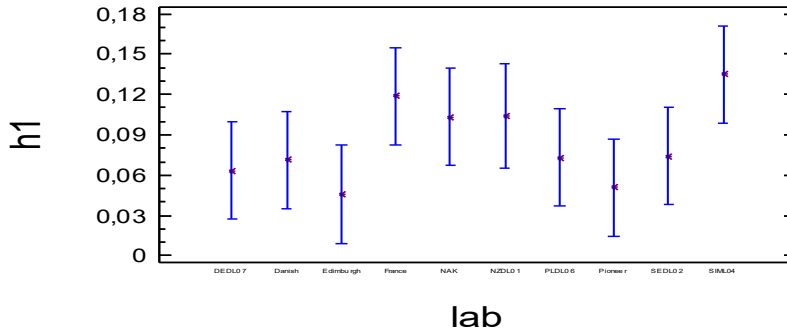
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**Figure 3.2.:**

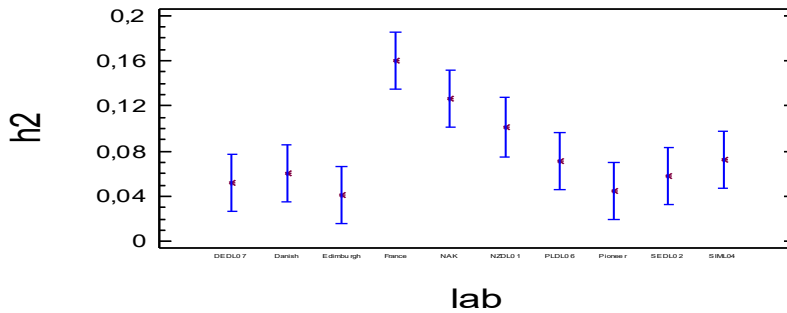
Variance of results per time in the oven 1-96hrs and per lab.

- X-axis: labs
- Y-axis: moisture content (%)
- The figure left of the y-axis indicates the consecutive readings in the time study (hours).

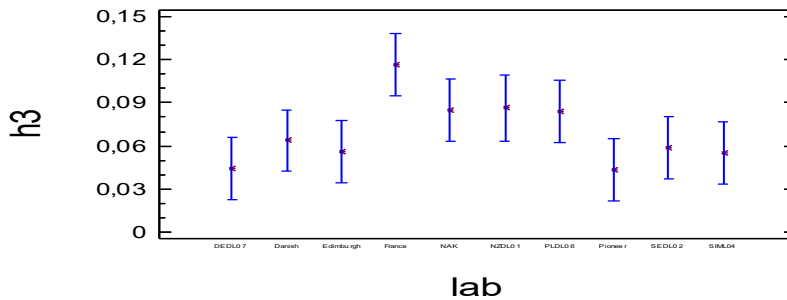
**Means and 95,0 Percent LSD Intervals**



**Means and 95,0 Percent LSD Intervals**



**Means and 95,0 Percent LSD Intervals**





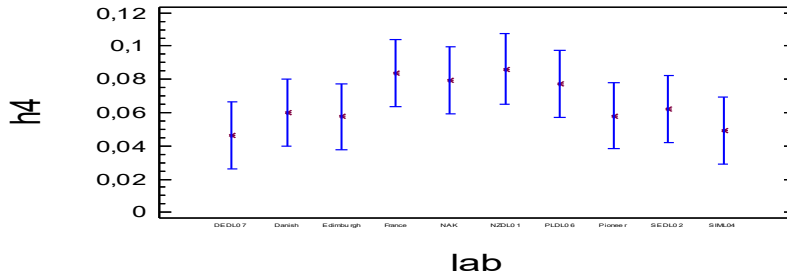


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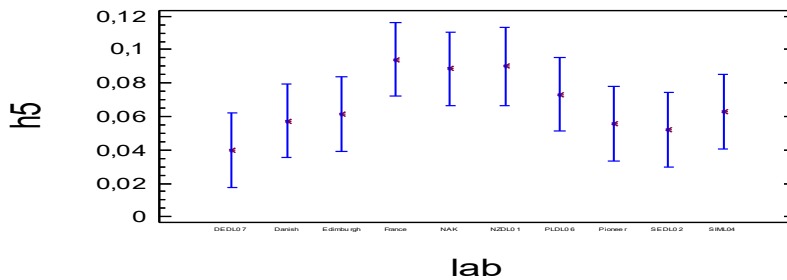
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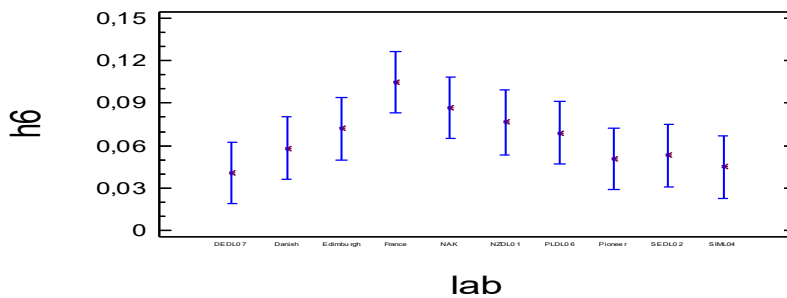
Means and 95,0 Percent LSD Intervals



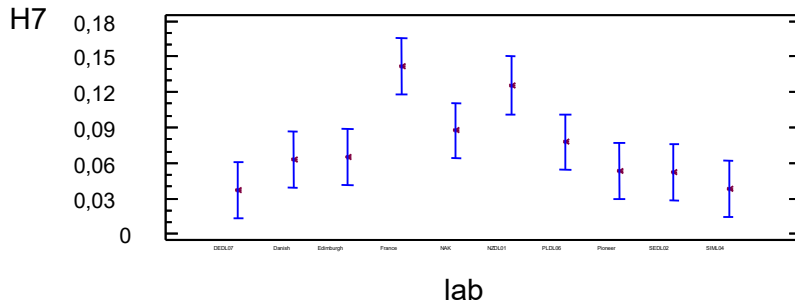
Means and 95,0 Percent LSD Intervals



Means and 95,0 Percent LSD Intervals



Means and 95,0 Percent LSD Intervals



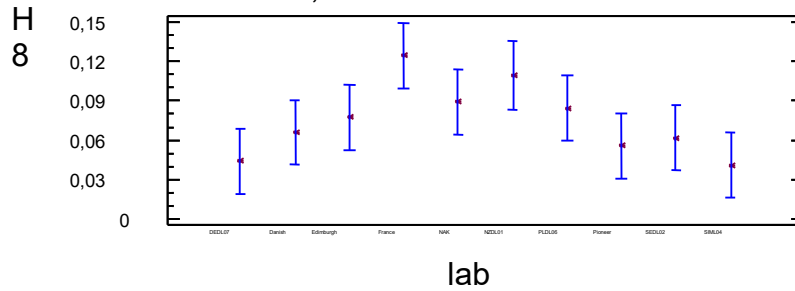


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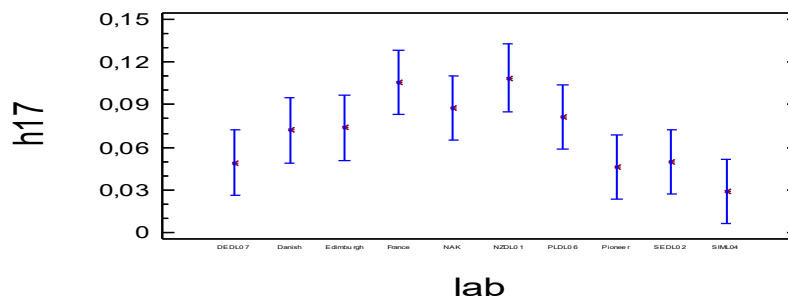
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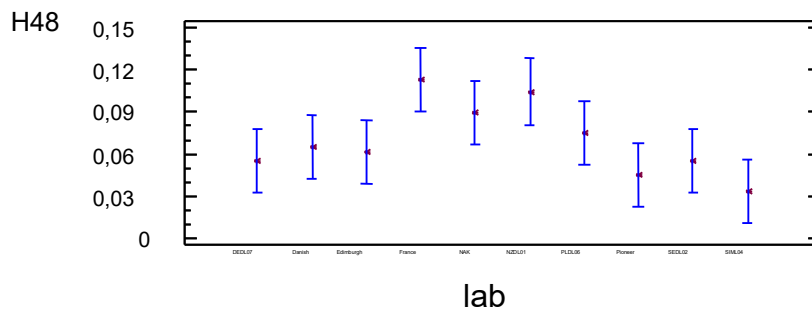
Means and 95,0 Percent LSD Intervals



Means and 95,0 Percent LSD Intervals



Means and 95,0 Percent LSD Intervals



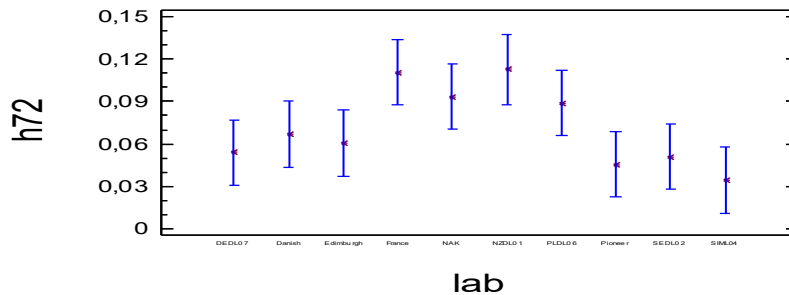


## INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)

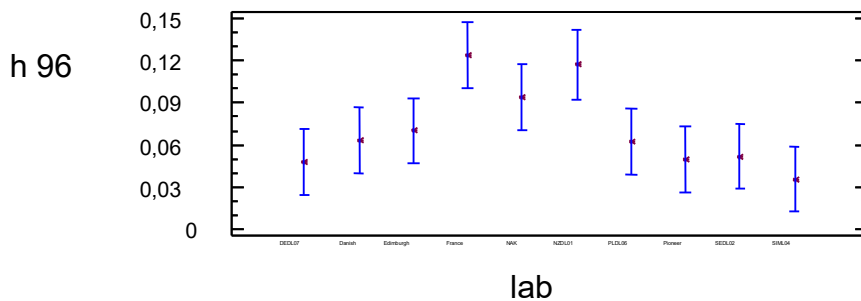
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### Means and 95,0 Percent LSD Intervals



### Means and 95,0 Percent LSD Intervals



From the graphs it is clear that the magnitude of the difference between two replicates averaged on all samples and methods, is quite consistent and comparable over time for the different laboratories. The median of the difference between two replicates per lab of all values is around 0.05% moisture content.

The 4<sup>th</sup> and 6<sup>th</sup> laboratories on the graph have a tendency to have a greater difference compared to other labs. These are lab numbers 7 and 10 in the following table (Table 3.2.)

**Table 3.2.:**  
LSD over all results per lab.

Method: 95,0 percent LSD

lab	Count	LS Mean	LS Sigma	Homogeneous Groups
3	40	0,0357849	0,0167251	X
9	40	0,0482489	0,0167251	XX
6	40	0,0496727	0,0167251	XX
8	40	0,0519167	0,0167251	XX
4	40	0,0624846	0,0167251	XX
2	40	0,0630419	0,0167251	XX
1	40	0,0700957	0,0167251	XXX
5	40	0,0937868	0,0167251	XXX
10	36	0,116963	0,0177274	XX
7	40	0,1239	0,0167251	X



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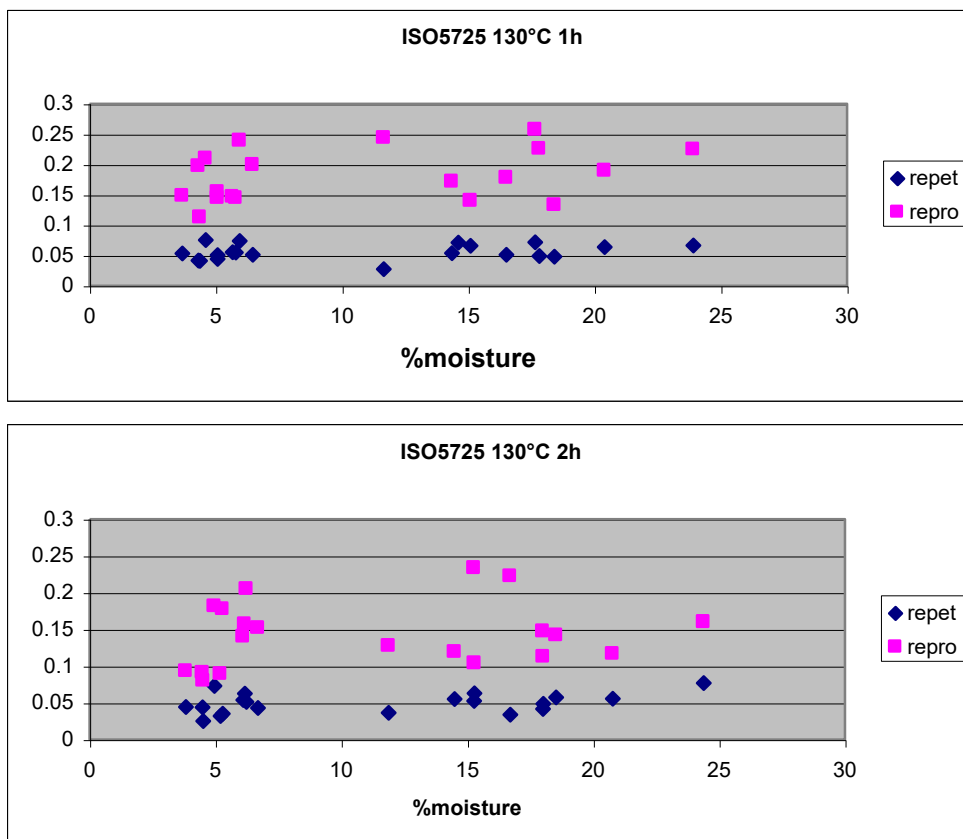
### 3.2. Repeatability and reproducibility (according to ISO 5725/2)

Repeatability (within lab variability) and reproducibility (between lab variability) were further analysed by means of ISO5725/2. In order to reduce the workload calculations were made only for a selected number of times from the time study and for the 17hrs103°C experiment. Values below 1 are considered as being satisfactory. The results (figures 3.3) indicate very good repeatability and reproducibility for this comparative testing. No general pattern indicating a link between moisture level and repeatability of reproducibility were observed. Often values increase at higher measured values which was not the case here. This may be due to the species confounded with moisture level but this was not investigated.

#### Figures 3.3:

Repeatability and reproducibility according to ISO5725 as affected by moisture content, for a selected part of the data.

On these graphs 20 values for repeatability or reproducibility are plotted, on the left the results from 10 species at the low level of moisture (less than 10%), and on the right the results from the 10 species at the high level of moisture (>10%). The results for the drying times, 1h 130°C, 2h 130°C, and 3h, 4h and 17 h at 103 °C are given.

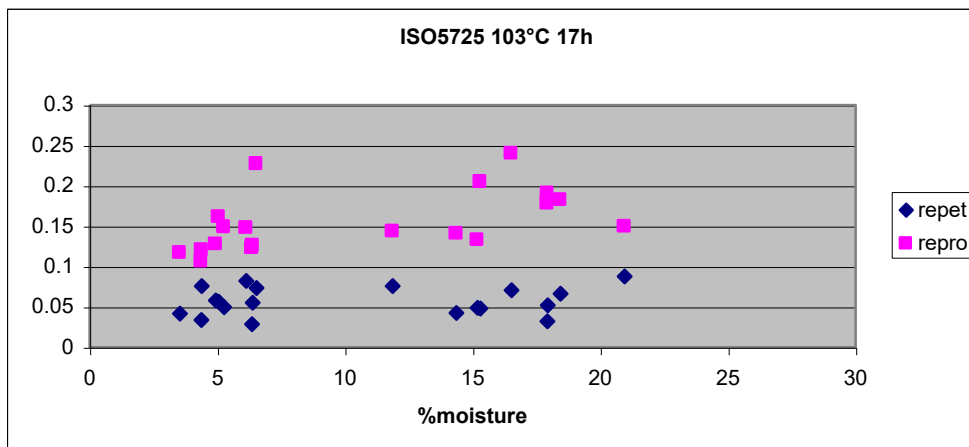
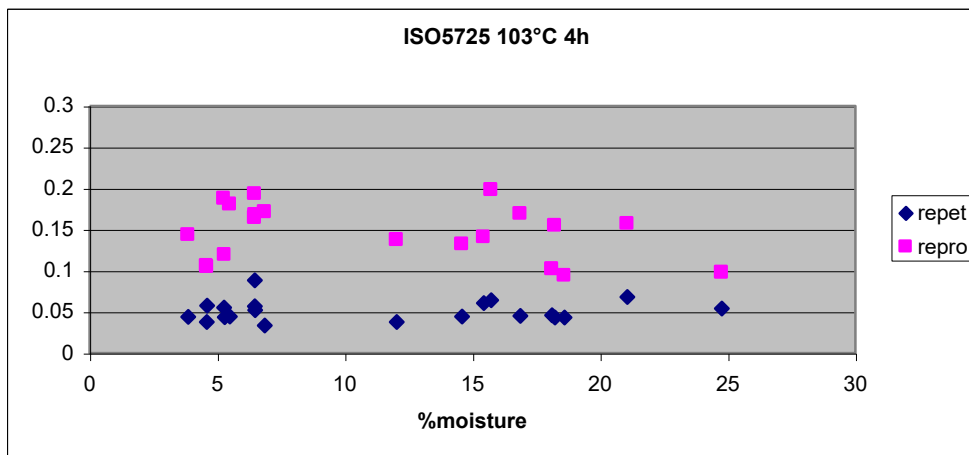
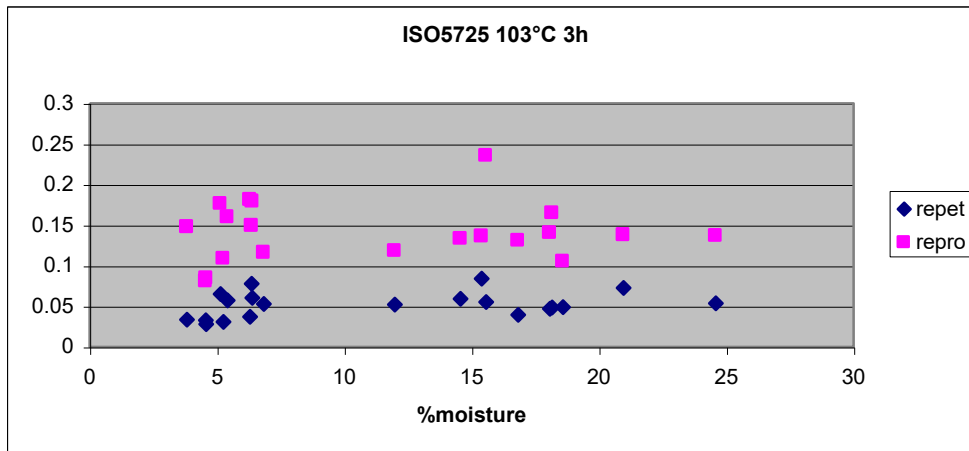




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In addition the reproducibility and repeatability per species and moisture level were calculated and found to give satisfactory results. Only *Brassica* at 130°C showed slightly higher, but still satisfactory results (figs 3.4).



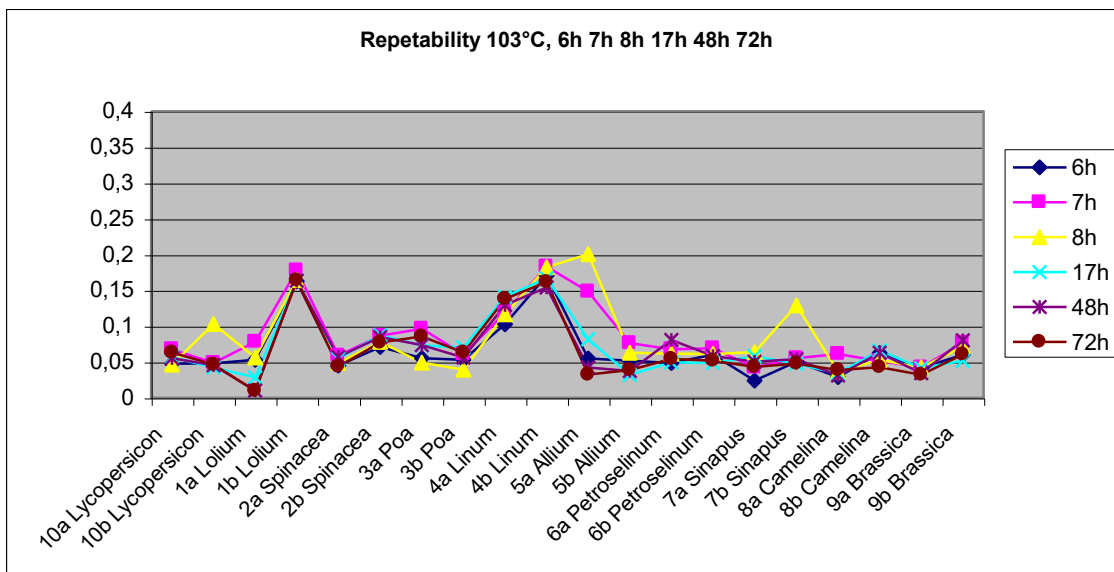
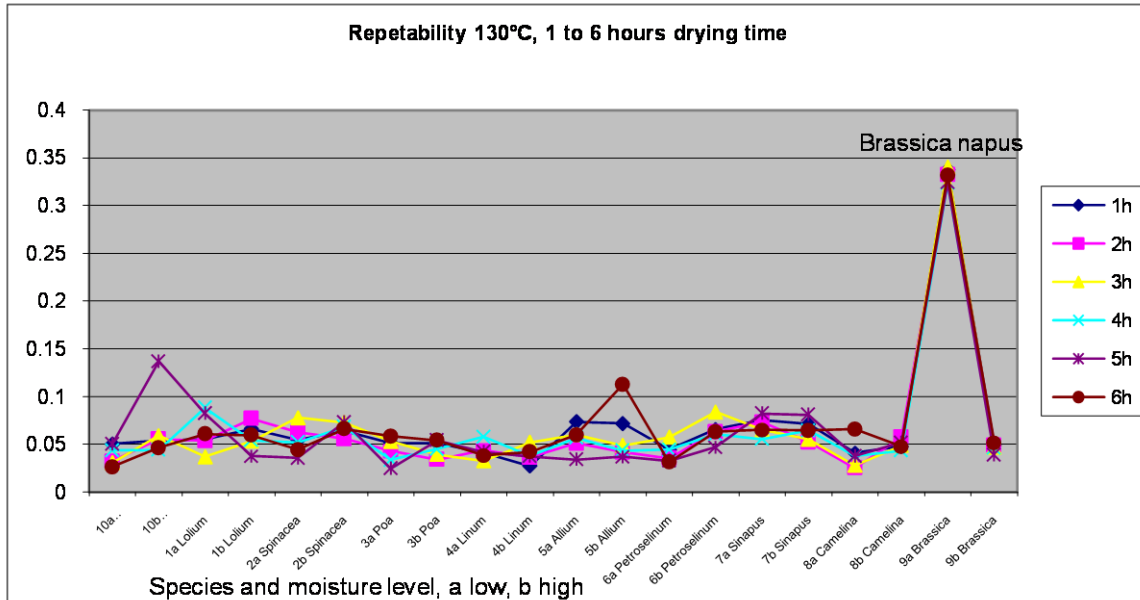
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**Figures 3.4:**

Repeatability and reproducibility according to ISO5725 per species, for a selected part of the data.

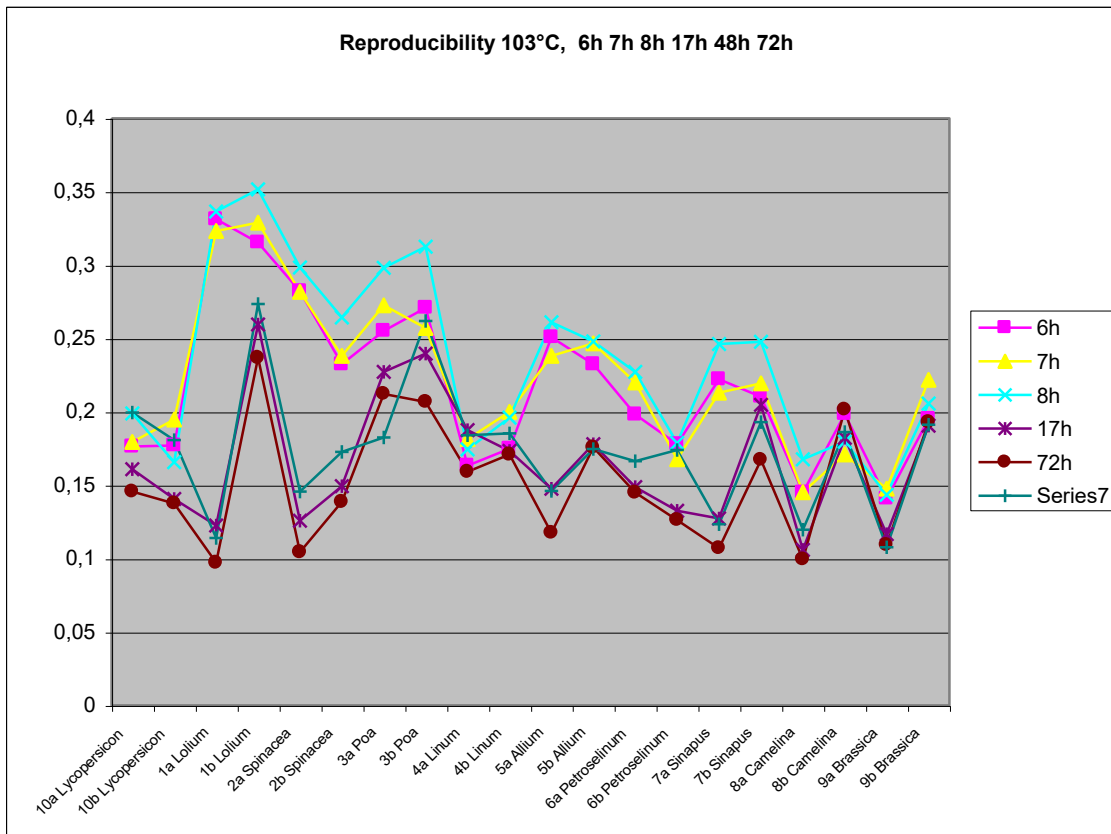
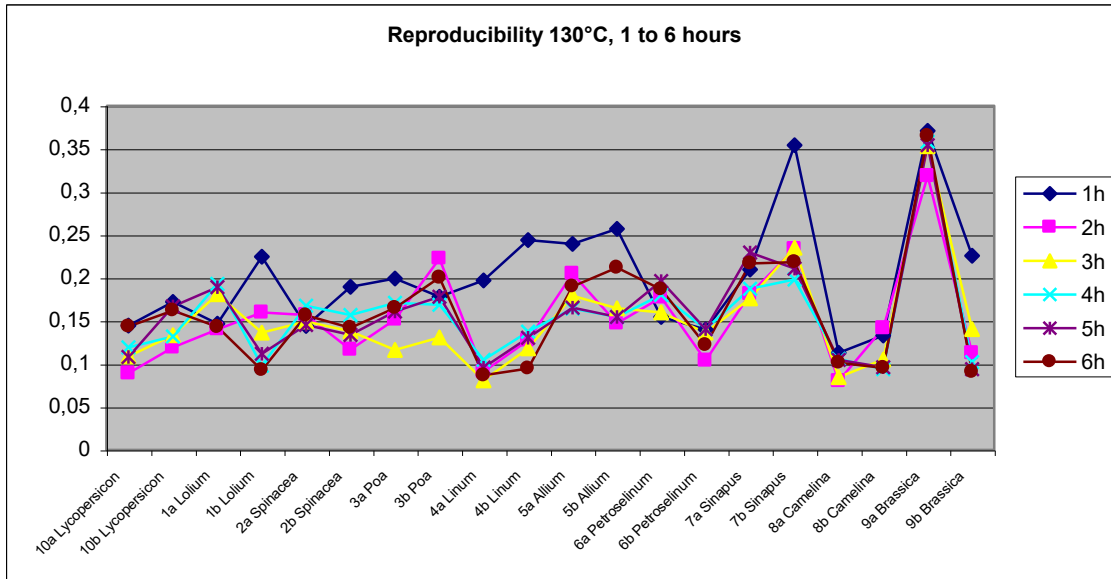




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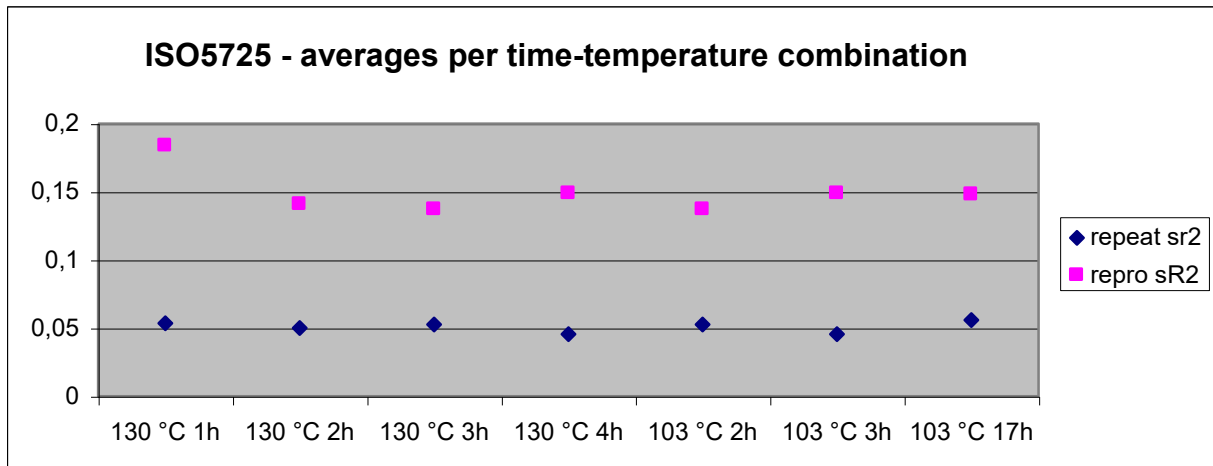
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**Figure 3.5:**

Repeatability and reproducibility according to ISO5725, averaged over all samples, for some combinations of time and oven temperature.



The general conclusion based on the last figure is that on average, one to six hours at 130°C on one hand and 17 to 72 hours at 103°C on the other hand have,

- similar repeatability values of around 0.05,
- similar reproducibility values of around 0.15 (fig. 3.5.).

In conclusion, repeatability and reproducibility are very similar for all durations and temperatures checked. Therefore other combinations of time and temperatures were not checked. They show the consistency of each lab response towards the average response

#### 4. Acknowledgments

The authors thank the representatives of all laboratories that participated in the comparative test: Craig McGill, Darja Vouk, Elzbieta Maluszynska, Gerarda de Boer – Raatgever, Johannes Dornhecker, Maria Laura Fusari, Maria Rosaria – Mannino, Ulf Kjellstrom, Gabriele Schillinger, Maria Angela Tillman.

Special thanks go to Jean-Louis Lafont of ISTA-STAT, for his advice on the statistical design of this comparative test and for doing statistical calculations.





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### 5. References

Don R. (2003). Moisture content proficiency test. *Seed Testing International*, **No.125**, 25-27.

Nijënstein, J.H., Nydam, J., Don, R. (in press-a). Oven seed moisture testing. I. Influence of oil content. *Seed Science and Technology*, .

Nijënstein, J.H., Nydam, J., Don, R. (in press-b). Oven seed moisture testing. II. Influence of temperature and test duration. *Seed Science and Technology*, .....

Nijënstein, J.H., Don, R., Nydam, J., (in press-c). Oven seed moisture testing. III. Methods compared. *Seed Science and Technology*, .....



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## ANNEX 1 – Z-SCORES

Z-scores	Total	LAB
LAB		LAB
1	4,492	1
5	0,086	5
3	1,392	3
X	9,898	X
4	-16,0454	
9	9,852	9
8	6,624	8
2	3,276	2
Z	-21,901Z	
7-4	-3,956	7-4
7-40	-7,166	7-40
6	-7,147	6
Y	40,515	Y
10	-19,91910	
Total	0,000	0,000

abs(Z-scores)	Total	LAB
1	6,806	1
5	7,732	5
3	5,437	3
X	11,887	X
4	16,045	
9	11,255	9
8	8,388	8
2	8,051	2
Z	21,901	Z
7-4	8,565	7-4
7-40	11,585	7-40
6	13,435	6
Y	41,305	Y
10	19,919	10



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Total 9,009 8,824 8,061 7,961 8,222 8,362 9,367 7,939 6,922 6,744 9,448 8,172 10,064 6,248 8,423 10,916 9,250 8,433 8,829 11,199

### trace of the computations of a sum of 20 z-scores

R : Copyright 2004, The R Foundation for Statistical Computing  
Version 1.9.1 (2004-06-21), ISBN 3-900051-00-3

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'citation()' on how to cite R in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or  
'help.start()' for a HTML browser interface to help.  
Type 'q()' to quit R.

```
>
>
> z1<-abs(rnorm(1000000))
> z2<-abs(rnorm(1000000))
> z3<-abs(rnorm(1000000))
> z4<-abs(rnorm(1000000))
> z5<-abs(rnorm(1000000))
```

quantile for sum of  
20 z-scores  
95% 20,6  
99% 22,7  
99,99% 27

conclusion:  
Brazil has  
unexpectedly high  
value

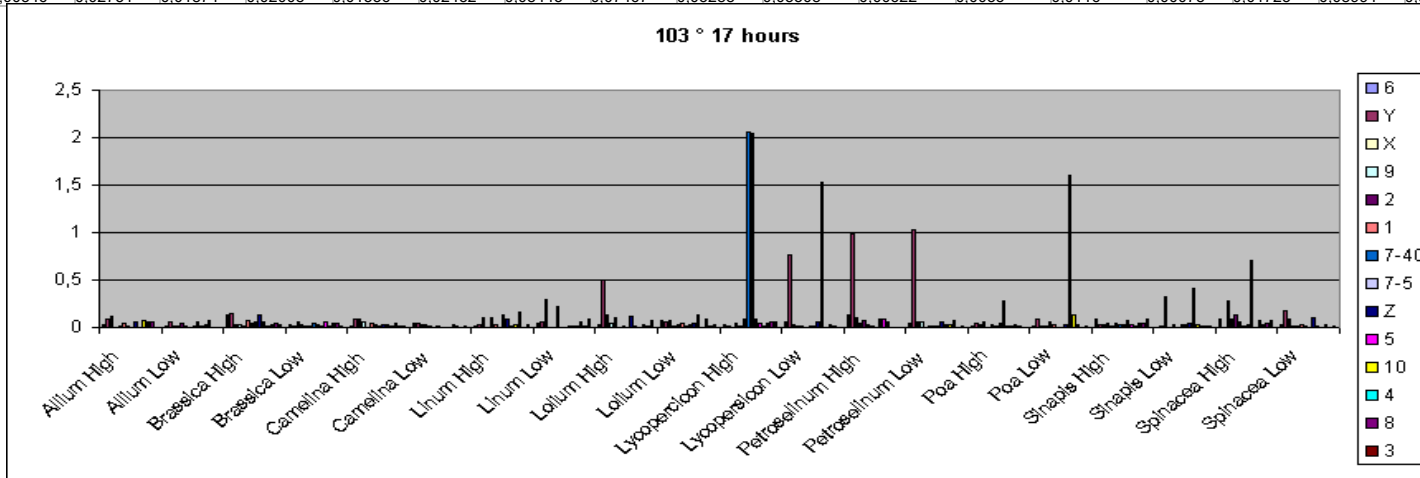


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## ANNEX 2 – STANDARD DEVIATIONS

lab	Allium High	Allium Low	Brassica High	Brassica Low	Camelina High	Camelina Low	Linum High	Linum Low	Lolium High	Lolium Low	Lycopersicon High	Lycopersicon Low	Petroselinum High	Petroselinum Low	Poa High	Poa Low	Sinapis High	Sinapis Low	Spinacea High	Spinacea Low
6	0,03437	0,01761	0,13039	0,02319	0,01421	0,04858	0,01252	0,04193	0,03543	0,06838	0,02772	0,05233	0,12622	0,04858	0,01018	0,01393	0,08443	0,00424	0,0874	0,02821
Y	0,0876	0,0577	0,144	0,0146	0,0822	0,0418	0,025	0,0572	0,502	0,0535	0,0141	0,763	0,9847	1,0315	0,0424	0,0868	0,0356	0,0163	0,0044	0,1756
X	0,1225	0,0126	0,0291	0,0602	0,0846	0,0366	0,0989	0,2931	0,135	0,0795	0,0018	0,0232	0,0974	0,06	0,0245	0,0143	0,0287	0,3298	0,276	0,0914
9	0,00184	0,01817	0,03521	0,02553	0,05565	0,03302	0,00516	0,00297	0,0379	0,01683	0,03981	0,00778	0,04745	0,06357	0,05636	0,02093	0,05013	0,00424	0,08407	0,01075
2	0,02001	0,0396	0,00863	0,01025	0,00049	0,00983	0,10988	0,00382	0,09843	0,03196	0,00884	0,01994	0,07927	0,00113	0,00608	0,0531	0,01167	0,02772	0,1364	0,02044
1	0,05028	0,01973	0,06788	0,01223	0,04893	0,00417	0,02934	0,22387	0,00615	0,04808	0,08902	0,00707	0,02404	0,00806	0,0321	0,02701	0,04653	0,00297	0,05735	0,02588
7-40	0,01167	0,00311	0,04228	0,04172	0,03069	0,0193			0,01874	0,0181	2,0581	0,00962	0,01973	0,01025	0,01846	0,00544	0,03401	0,03062	0,00919	0,02051
7-5	0,00233	0,01195	0,05643	0,02758	0,01655	0,00516	0,13506		0,0046	0,02489	2,04474	0,01054	0,0058	0,01068	0,04702	0,00552	0,03415	0,03062	0,02341	0,00629
Z	0,0537	0,063	0,1275	0,0091	0,0313	0,0032	0,0836	0,0095	0,1187	0,0513	0,0841	0,0624	0,0833	0,0547	0,2815	0,0338	0,0726	0,0423	0,7079	0,1019
5	0,00361	0,02107	0,06053	0,05367	0,02291	0,00587	0,0198	0,01351	0,02093	0,13937	0,04405	1,52643	0,08167	0,02609	0,0094	1,60549	0,03359	0,4097	0,00594	0,01867
10	0,07764	0,03557	0,01506	0,01513	0,01945	0,0355	0,02567	0,01747			0,00884	0,00332	0,06505	0,03359	0,01414	0,12869	0,01351	0,02623	0,07212	0,00559
4	0,05431	0,06682	0,03387	0,03818	0,04695	0,01096	0,16094	0,05819	0,02291	0,08938	0,03882	0,03627	0,00085	0,0746	0,02539	0,03345	0,04228	0,02199	0,03147	0,0338
8	0,05155	0,00127	0,04214	0,04709	0,021	0,00629	0,00021	0,01245	0,01146	0,00735	0,06074	0,00771	0,00071	0	0,02164	7,1E-05	0,04115	0,02065	0,0449	7,1E-05
3	0,00085	0,00346	0,02751	0,01874	0,02008	0,01336	0,02432	0,08443	0,07467	0,03288	0,05508	0,00622	0,0053	0,0116	0,00078	0,01725	0,08994	0,01442	0,0664	0,01853

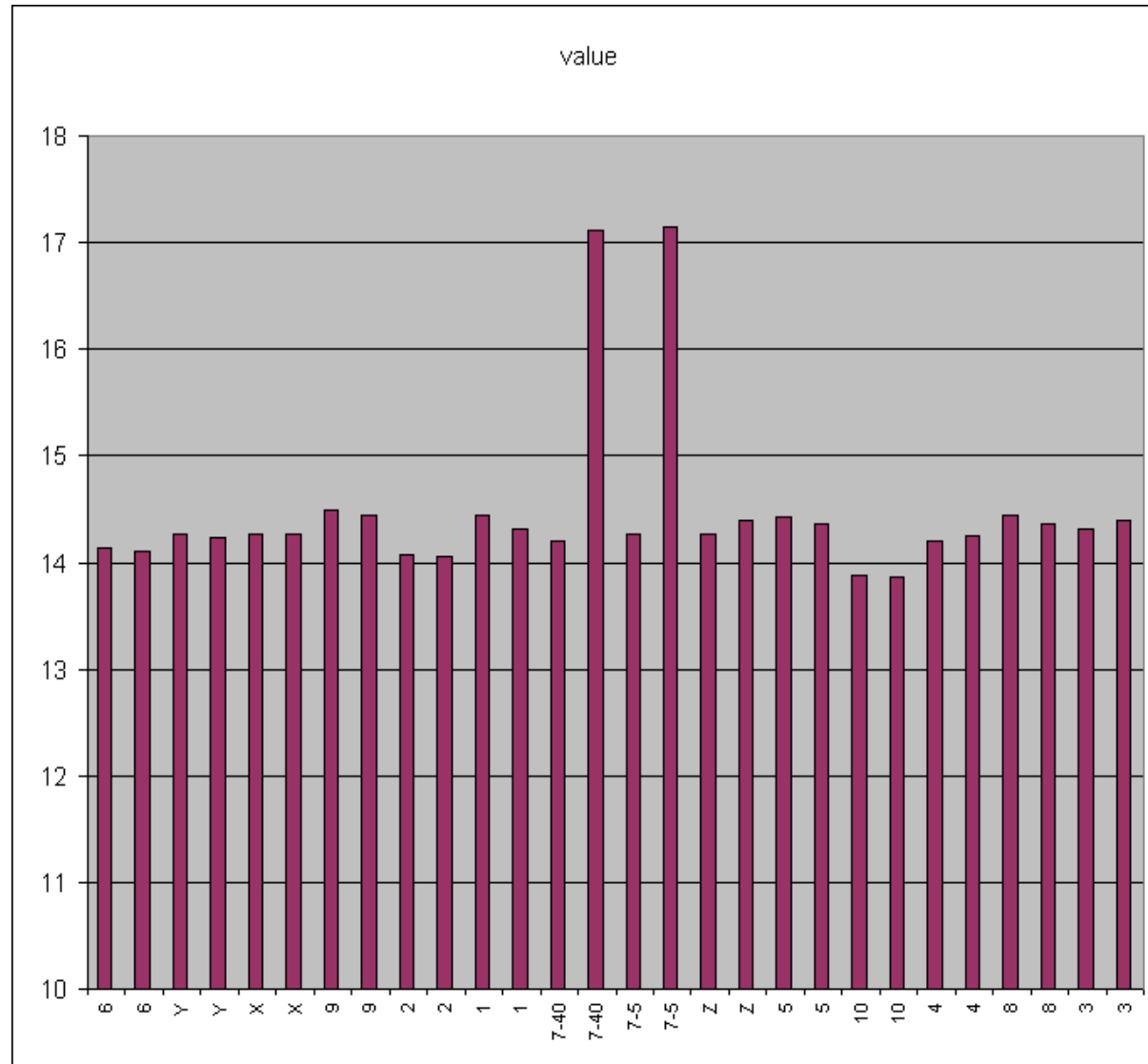




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lab	sample	rep	Value
6	Lycopersicon High 1		14,1401
6	Lycopersicon High 2		14,1009
Y	Lycopersicon High 1		14,26
Y	Lycopersicon High 2		14,24
X	Lycopersicon High 1		14,2708
X	Lycopersicon High 2		14,2734
9	Lycopersicon High 1		14,4956
9	Lycopersicon High 2		14,4393
2	Lycopersicon High 1		14,0714
2	Lycopersicon High 2		14,0589
1	Lycopersicon High 1		14,4422
1	Lycopersicon High 2		14,3163
7-40	Lycopersicon High 1		14,199
7-40	Lycopersicon High 2		17,1096
7-5	Lycopersicon High 1		14,2595
7-5	Lycopersicon High 2		17,1512
Z	Lycopersicon High 1		14,2687
Z	Lycopersicon High 2		14,3876
5	Lycopersicon High 1		14,4307
5	Lycopersicon High 2		14,3684
10	Lycopersicon High 1		13,873
10	Lycopersicon High 2		13,8605
4	Lycopersicon High 1		14,1984
4	Lycopersicon High 2		14,2533
8	Lycopersicon High 1		14,4431
8	Lycopersicon High 2		14,3572
3	Lycopersicon High 1		14,3089
3	Lycopersicon High 2		14,3868

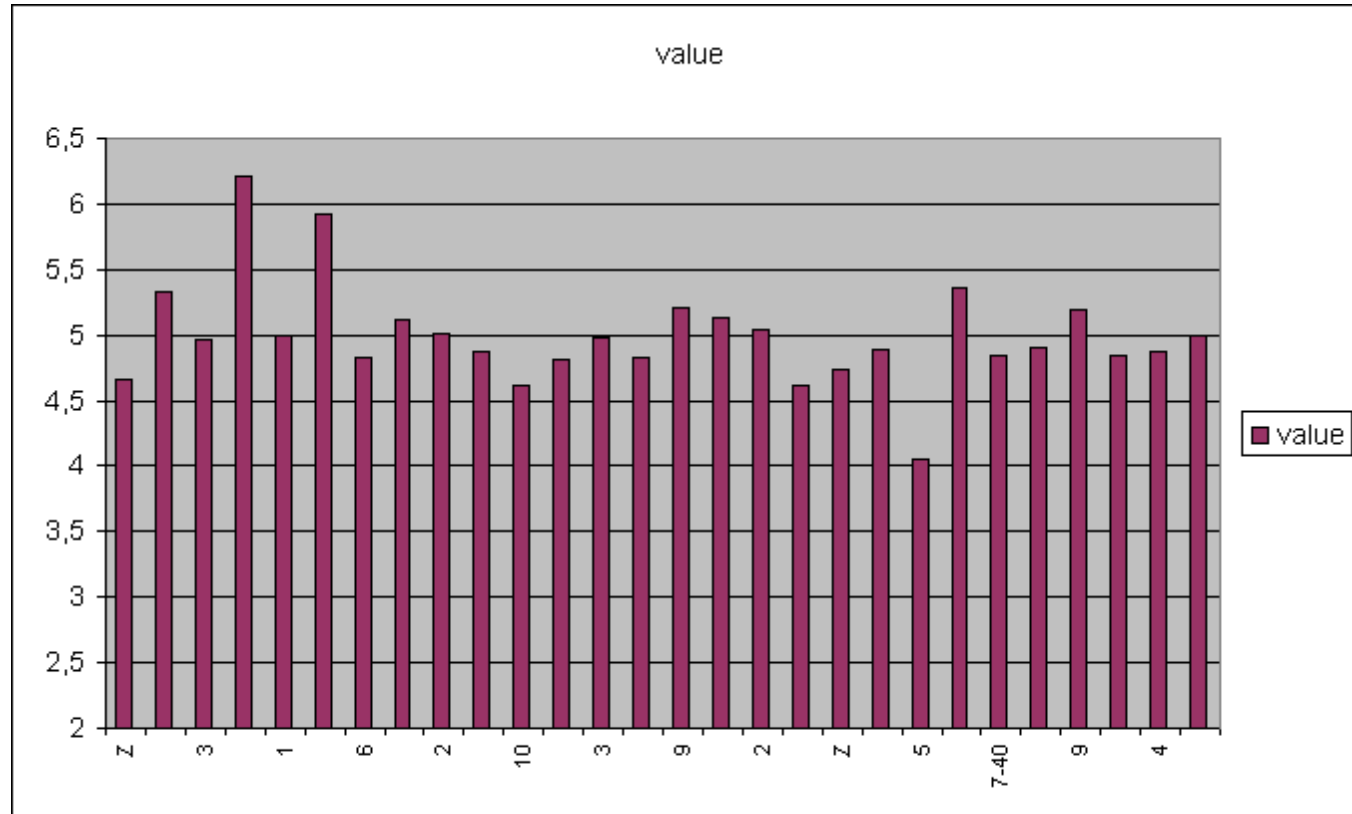




# INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)

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lab	sample	rep	Value
Z	Lycopersicon Low 1		4,655
X	Lycopersicon Low 2		5,3228
3	Lycopersicon Low 2		4,9666
NAK	Lycopersicon Low 2		6,2053
1	Lycopersicon Low 2		4,99
Y	Lycopersicon Low 1		5,92
6	Lycopersicon Low 1		4,835
8	Lycopersicon Low 1		5,1225
2	Lycopersicon Low 1		5,0115
7-5	Lycopersicon Low 1		4,8677
10	Lycopersicon Low 1		4,6096
4	Lycopersicon Low 2		4,8153
3	Lycopersicon Low 1		4,9754
7-40	Lycopersicon Low 1		4,8275
9	Lycopersicon Low 2		5,2038
8	Lycopersicon Low 2		5,1334
2	Lycopersicon Low 2		5,0397
10	Lycopersicon Low 2		4,6143
Z	Lycopersicon Low 2		4,7433
7-5	Lycopersicon Low 2		4,8826
5	Lycopersicon Low 1		4,0466
X	Lycopersicon Low 1		5,3556
7-40	Lycopersicon Low 2		4,8411
6	Lycopersicon Low 2		4,909
9	Lycopersicon Low 1		5,1928
Y	Lycopersicon Low 2		4,841
4	Lycopersicon Low 1		4,8666
1	Lycopersicon Low 1		5

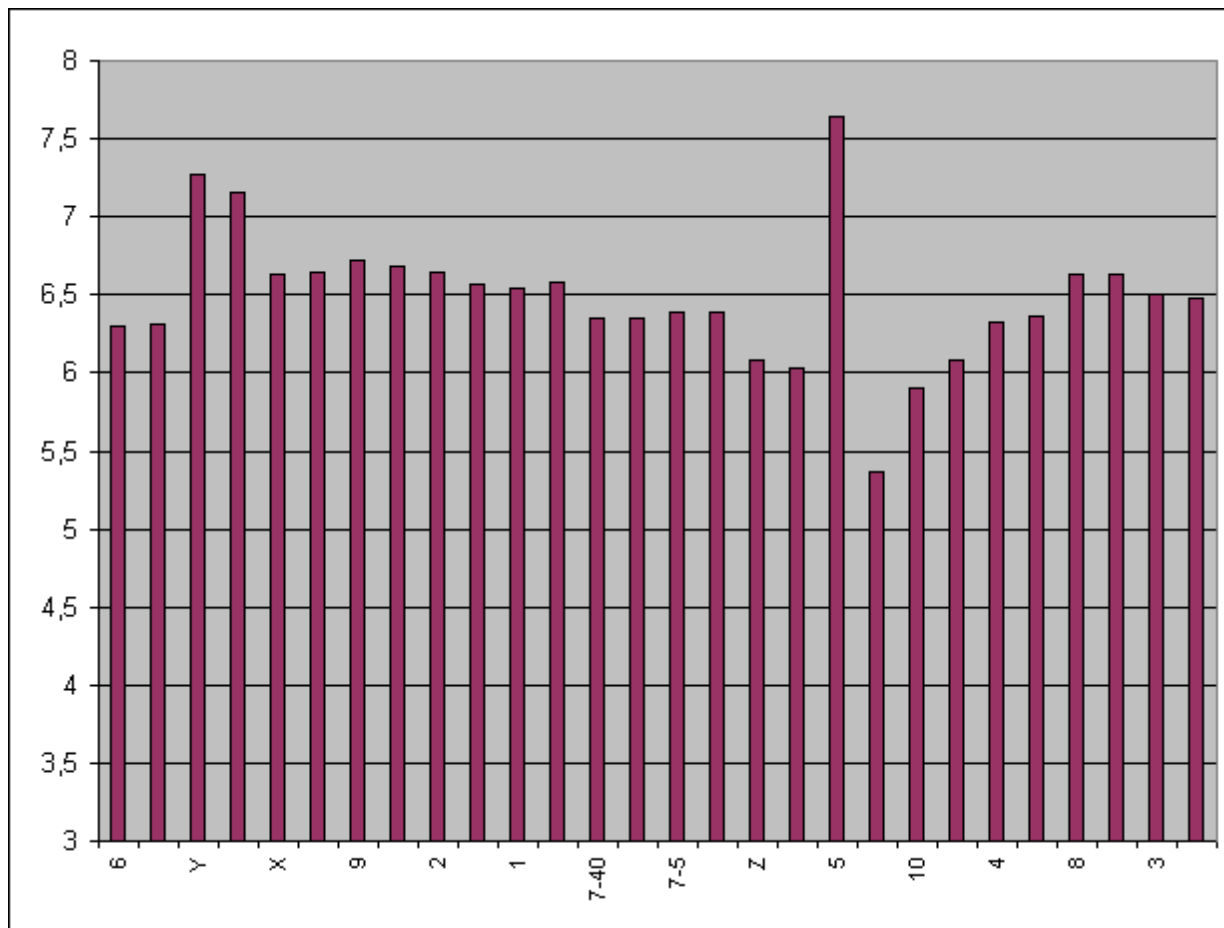




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lab	sample	rep	Value
6	Poa Low	1	6,2956
6	Poa Low	2	6,3153
Y	Poa Low	1	7,2771
Y	Poa Low	2	7,1543
X	Poa Low	1	6,626
X	Poa Low	2	6,6462
9	Poa Low	1	6,7166
9	Poa Low	2	6,687
2	Poa Low	1	6,6429
2	Poa Low	2	6,5678
1	Poa Low	1	6,5429
1	Poa Low	2	6,5811
7-40	Poa Low	1	6,3524
7-40	Poa Low	2	6,3447
7-5	Poa Low	1	6,3929
7-5	Poa Low	2	6,3851
Z	Poa Low	1	6,0823
Z	Poa Low	2	6,0345
5	Poa Low	1	7,6386
5	Poa Low	2	5,3681
10	Poa Low	1	5,9022
10	Poa Low	2	6,0842
4	Poa Low	1	6,3202
4	Poa Low	2	6,3675
8	Poa Low	1	6,6292
8	Poa Low	2	6,6291
3	Poa Low	1	6,5015
3	Poa Low	2	6,4771





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### ANNEX 3:

An example of 'crossing lines' for labs X and Y and deviating results of lab Z. All results of these three labs were discarded.

Poa high 130°C

