Open-Bio – Opening bio-based markets via standards, labelling and procurement: Deliverable 3.1: Performance characteristics for horizontal bio-based carbon content standard – round robin assessment results

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Work package 3

Bio-based content

Deliverable N° 3.1:

Performance characteristics for horizontal bio-based carbon content standard – round robin assessment results

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1 Publishable summary

This report presents the results of the round robin assessment that was organised with the aim to investigate the performance characteristics of the method that is described in CEN TS 16640 for the bio-based carbon content determination, in order to convert the available technical specification into the European standard. The round robin assessment was initiated in the framework of the European Open-Bio project (www.biobasedeconomy.eu). The assessment involved 11 independent laboratories to whom in total 132 samples were delivered (11 equivalent sets of samples, 12 samples each set).

Next samples were involved in the round robin testing:

Sample 1. White water soluble matt paint, volatile components about 34% are present; possible difficulties with ignition, combustion in an elemental analyzer is recommended. Non-hazardous.
Sample 2. White emulsion; non-volatile; non-hazardous; used as one of components of a sun lotion.
Sample 3. White emulsion; non-volatile; non-hazardous; used as one of components of a sun lotion (different from Sample 2)
Sample 4. A wheat straw panel, 10cm x 10cm; non-hazardous; can be used for different construction and building purposes
Sample 5. Highly flammable liquid (biodiesel); used as a fuel.
Sample 6. A container filled with bio-gas, pressurized to 2.5bar, H_2S content 25ppm. The biogas contain approximately 60% of CH_4 and 40% CO_2.
Sample 7. White surfactant granules that are used in cosmetics; non-hazardous.
Sample 8. Cosmetic emulsion with high water content; non-hazardous.
Sample 9. Multilayer packaging film; presents no hazard.
Sample 10. Silk paint; non-hazardous.
Sample 11. Bio-based binder used in paints; non-hazardous.
Sample 12. Wooden particle board ground to 0.5mm; presents no hazard.
None of these samples demanded a special storage conditions.

These samples, together with the latest available version of CEN TS 16640, were sent to each participating laboratory.

Below the **list of participating laboratories** is presented:

Agroisolab GmbH, Germany
Beta Analytic, USA
Centre de Datation par le RadioCarbone/Institute of Analytical Sciences, France
Energy research Center of the Netherlands (ECN), the Netherlands

SGS, France
SKZ, Germany
Silesian University of Technology, Institute of Physics, Radiocarbon Laboratory, Poland
Scion/GNS Science, National Isotope Centre, Rafter Radiocarbon, New Zealand
University of Wageningen, Food and Bio-based Research, the Netherlands
University of Groningen, Center for Isotope Research (CIO), the Netherlands
University of York, Green Chemistry Centre of Excellence, United Kingdom

Due to the confidentiality agreements, the results obtained by each laboratory are presented in anonymous way. Every laboratory was prescribed a name known only to the organiser of the assessment and to that specific laboratory. In the final report, the results are presented using these names (Lab 1, Lab 2, … Lab 11) so that every laboratory can have an overview of all results, but is able to recognise only its own results. Laboratories were free to choose their own method when preparing a (sub)sample that would be homogeneous and representative of the received sample. For pre-treatment, CEN TS 16640 was advised to follow.

The round robin test was carried out to determine the influence of parameters which may vary between individual laboratories. Subsequently, the reproducibility standard deviations were calculated based on the results reported by each laboratory. Statistical evaluation of the results was done when analysing the results from all participating laboratories on each individual sample. Extremely biased results were investigated for possible errors. In the current study, the Grubbs test was used for statistical evaluation of the results that were reported for each sample by each laboratory. Outliers and stragglers that were defined based on the results of Grubbs analysis, were excluded from the calculations of measured average numbers and the reproducibility standard deviations among all laboratories.

The results of performed assessment showed a good consistency. The maximum number of outliers/stragglers (1 outlier, 1 straggler) when analysing the reported results on the 14C content, was observed for Sample 1 that was a paint with low carbon content and with a high volatile fraction (approximately 35%). This can be related to the combustion difficulties and possible loss of carbon that could be present in the volatile fraction. The maximum value for the variation of the coefficient of the reproducibility (17.7%) for the biogenic carbon content was observed for the same Sample 1 (10.2 ± 1.8 % of 14C as fraction of total carbon, see Table 5), that was one of the most challenging samples. Analysing the calculated performance characteristics for the total carbon content, one can observe that the highest value for the variation of the reproducibility standard deviation for total carbon content was 8.8% (15.9 ± 1.47) for Sample 8 that was cosmetic emulsion with high water content (see Table 3). Relatively high variation in the coefficient of the reproducibility for Sample 1 and Sample 8 can be caused by combustion difficulties of these two samples: some laboratories used combustion enhancers and some did not. This can explain somewhat high values for the reproducibility variations and has to be taken into account when converting paint-like or water-containing samples into carbon dioxide.

For the C14 analysis, the known LSC (Liquid Scintillation Counting)or the AMS (Accelerated Mass Spectrometry) techniques were used in this round robin assessment. 3 of 11 laboratories did the 14C analysis using the LSC method (no direct LSC was performed on any samples). By 8 laboratories the AMS analysis was used in order to determine the 14C amount in the delivered samples. The results of the round robin assessments indicated that these two techniques give the equivalent results as no inconsistencies were observed for the results of the measurements when using AMS (Accelerated Mass Spectrometry) and LSC (Liquid Scintillation Counting) techniques.

2 Introduction

2.1 The goal and organisation of the round robin assessment

Generally round robin assessments are organized to ensure the quality and reproducibility of measurement results and/or the same test methods used by different laboratories. Reproducibility cannot be guaranteed if the laboratories get very different results in the analysis of identical samples. Reliable information on method accuracy and laboratory performance depends on the limit of participants. Minimal number of participating laboratories is 8.

A round robin test is performed on identical samples which are sent to the participating laboratories which use the agreed methods of analysis. Typically the samples are from an institution that conducts the trial and invites the laboratories to participate.

A round robin test usually determines the influence of parameters which may vary between individual laboratories, and it does not represent a substitute for the calibration procedure. All tests shall be performed under repeatability conditions. Statistical evaluation of the results is done when analysing the results from all participating laboratories. Extremely biased results have to be investigated for possible errors.

The aim of the round robin assessment that is reported in this document, was to determine total carbon content and the bio-based carbon content of different types of materials or products in order to ensure the validity of the method that is proposed to be used in the horizontal standard for determination of the bio-based carbon content (CEN TS 16640).

The number of participating laboratories in given assessment was 11. Due to the confidentiality agreement each laboratory is mention in this report in anonymous manner (Lab 1, Lab 2, Lab3, … Lab 11).
Accordingly to the goal of the study, each participating laboratory was asked to determine:

a) Total carbon content and combustion recovery
b) Biogenic carbon content (C14)

Since the method described in CEN TS 16640 shall be applicable to any products, the selection of samples for the round robin tests was done to cover as much as possible different and challenging products. 12 different samples including emulsions, liquid, solid and gaseous samples from different suppliers were distributed to each laboratory. In total 132 samples were distributes. A brief characteristic of the samples is given in next sections of this report. Technical specification CEN/TS 16640 (Bio-based products – Determination of the bio-based carbon content of products using the radiocarbon method) was advised as a guideline. Besides, each laboratory was supplied with additional document that explained in details what kind of analysis was necessary for each product.

After the results of the measurements were reported, the Grubbs test was used for statistical evaluation of the results on each sample. Outliers and stragglers that were defined based on the results of Grubbs analysis, were excluded from calculations of measured average numbers and reproducibility standard deviations among all laboratories.

Next paragraphs give a brief description of each sample and present the summarizing overview of the results on the total carbon content and on the biogenic carbon content. Performance characteristics (measured average for each sample, reproducibility standard deviation and coefficient of the variation of the reproducibility) are presented both for total carbon content and for the biogenic carbon content, for each of analysed samples. More detailed reports on each individual sample are given in Appendix A (for total carbon content for each of Samples 1-12) and in Appendix B (for biogenic carbon content for each of Samples 1-12). Appendices A and B also present the Z-score plots for each individual sample. For a given sample, these plots illustrate the deviation of the results of each single laboratory from the calculated average.

2.2 Bio-based carbon content determination accordingly to CEN TS 16640

As it was already mentioned earlier in this report, CEN TS 16640 describes the method for the bio-based carbon content determination in a wide range of material or product. Therefore selection of samples for the round robin tests was done to cover as much as possible different and challenging products. The proposed method is based on complete combustion of a sample and capturing of the CO_2 gas with the subsequent titration in order to determine the total carbon concentration.

Total carbon content of each sample can be determined in two ways: 1 - from the carbon dioxide that is formed during combustion and subsequently trapped into a washing bottle containing a sodium hydroxide solution or absorbent column. The sodium hydroxide solution is titrated with acid to determine the carbonate concentration. From this, total carbon concentrations can be calculated; 2 - using an elemental analyser. The recovery of the combustion is calculated as a ratio between the carbon content determined from titration to the carbon content determined via elemental analyser. Generally, the recovery rate should be at least 90%, as it was already reported in Deliverable 3.4 of KBBPPS.

As it is described in CEN TS 16640, the pre-treatment (combustion) can be done in a number of ways: calorimetric bomb, or in a tube furnace, or in a laboratory scale combustion apparatus:

- Calorimetric bomb.
  When combustion is done in a calorimetric bomb, the carbon dioxide formed is subsequently led into a washing bottle containing a sodium hydroxide solution or through a cartridge containing a solid absorbent (e.g. Ascarite). From the solid absorbent the carbon dioxide is washed of into a sodium hydroxide solution. The sodium hydroxide

solution is titrated with acid to determine the carbonate concentration. As an example, in one of the laboratories, the material is combusted with pure oxygen (30psi) in a closed steel container. The temperature inside the closed container can reach up to >1500°C. Combustion in a calorimetric bomb cannot be done for gaseous samples.

- Element analyser.
  An element analyser can be used for combustion as well. In an elemental analyser of one of the laboratories, the material is combusted (975°C) in a quartz tube containing chromium oxide, copper wires, and silvered cobaltous oxide with oxygen and helium carrier gasses. The carbon dioxide formed is collected in a washing bottle containing a sodium hydroxide solution or collected in a cartridge containing a solid absorbent (e.g. Ascarite). The sodium hydroxide solution is titrated with acid to determine the carbonate concentration. As an advantage the elemental analyser can also be used for the determination of the total carbon-, hydrogen-, nitrogen- and oxygen content of the material.
- Tube furnace.
  A tube furnace with temperature controller capable of maintaining a stable furnace temperature of 1100°C and a quartz tube can be used for combustion. The inlet end of the quartz tube shall be large enough to accept a sample boat and to have side arms for introduction of oxygen and inert gas. The construction is such that the carrier gas sweep (200 ml/min oxygen plus 200 ml/min argon) the inlet zone transporting all of the volatilized sample into a high-temperature oxidation zone. The reaction product (carbon dioxide) is collected at the outlet of the quartz tube in a washing bottle containing a sodium hydroxide solution or in a cartridge containing a solid absorbent (e.g. Ascarite). The sodium hydroxide solution is titrated with acid to determine the carbonate concentration.

In this round robin assessment, all participating laboratories performed the total carbon analysis using an elemental analyzer. The results are presented in the next paragraphs.

For the C14 analysis, a number of ways can be used: Atomic Mass Spectroscopy (AMS), Liquid Scintillation Counting (LSC), or direct Liquid Scintillation Counting. **The AMS method** determines the presence of 14C directly: the atoms in the sample are converted into a beam of ions, then the formed ions are accelerated in an electric field, deflected in a magnetic field and detected in ion detectors resulting in the determination of the relative isotope abundances of these ions. As the 14C is determined in graphite (carbon), all the carbon in the samples has to be converted into graphite before analysing. With AMS, the modern fraction in the carbon, present in the sample, is determined. The total carbon content is not determined with this technique and shall be determined separately. **The LSC method** determines the isotope abundance of 14C indirectly, through its emission of beta-particles due to the radioactive decay of the 14C atoms. The beta-particles are detected through their interaction with scintillation molecules. The number is scintillations is counted and is proportional to the 14C amount in a sample. Only for products that are homogeneous liquids, in some cases **direct**

LSC measurement with the LSC technique is possible, when a liquid sample can be directly mixed with the scintillation liquid without prior combustion. This option is only allowed if equivalence with the methods with conversion to CO_2 can be demonstrated. This will in general be the case if no quenching is observed, or if correction for quenching is performed using standard addition technique using the same, 14C labelled, bio-based product with known 14C activity.

For the C14 analysis, the LSC or the AMS techniques were used in this round robin assessment. 3 of 11 laboratories did the 14C analysis using the LSC method. No direct LSC was performed on any samples. By 8 laboratories the AMS analysis was used in order to determine the 14C amount in the delivered samples.

3 Description of samples

Below is the list of samples, with the description that was supplied to the laboratories. None of samples demanded a special storage conditions.

Sample 1	White water soluble matt paint, volatile components about 34% are present; possible difficulties with ignition, combustion in an elemental analyser is recommended. Non-hazardous.
Sample 2	White emulsion; non-volatile; non-hazardous; used as one of components of a sun lotion.
Sample 3	White emulsion; non-volatile; non-hazardous; used as one of components of a sun lotion (different from Sample 2).
Sample 4	A wheat straw panel, 10cm x 10cm; non-hazardous; can be used for different construction and building purposes.
Sample 5	Highly flammable liquid (biodiesel); used as a fuel.
Sample 6	A container filled with bio-gas, pressurized to 2.5bar, H_2S content 25ppm. The biogas contains approximately 60% of CH_4 and 40% CO_2.
Sample 7	White surfactant granules that are used in cosmetics.
Sample 8	Cosmetic emulsion with high water content.
Sample 9	Multilayer packaging film.
Sample 10	Silk paint.
Sample 11	Bio-based binder used in paints.
Sample 12	Wooden particle board ground to 0.5mm.

4 Grubbs test and Z-score analyses

Grubbs test

In the current study, the Grubbs test was used for the statistical evaluation of the results that were reported for each sample by every participating laboratory.

This test is used to detect the outliers and/or stragglers. The Grubbs test always checks the value whether the extreme value (high or low) that shows the largest absolute deviation from the mean, is an outlier or a straggler. In the current study, the tested data were the minimum and maximum measured values reported by all participating laboratories for each of the samples.

The application of the test is the following:

- the maximum (X_{max}) and the minimum (X_{min}) among the reported measured values have to be determined.
- The average among all measured values X_{mean} (for the same sample) and the reproducibility standard deviation (SD) have to be calculated.
- Then the ratio $|X_{min} – X_{mean}|/SD$ and $|X_{max} – X_{mean}|/SD$ is calculated and the results are compared to the critical values given by the Grubbs table (see Table 1). If for a given number of measurement, the resulting value is greater than the critical value, then the corresponding minimal (or maximum) value can be regarded as an outlier or a straggler, depending on the reliability interval. An observation is considered an outlier if the reliability is 99%. For stragglers the limit of 95% reliability applies.

Table 1. Critical values for the Grubbs test depending on the number of measurements.

GRUBBS TABLE		
No of measurements	Critical values	
	1% - outlier	5% - straggler
3	1.155	1.155
4	1.496	1.481
5	1.764	1.715
6	1.973	1.887
7	2.139	2.020
8	2.274	2.126
9	2.378	2.215
10	2.482	2.290
11	2.564	2.355
12	2.636	2.412
13	2.699	2.462
14	2.755	2.507
15	2.806	2.549
16	2.852	2.585
17	2.894	2.620
18	2.932	2.651
19	2.968	2.681
20	3.001	2.709
21	3.031	2.733
22	3.060	2.758
23	3.087	2.781
24	3.112	2.802
25	3.135	2.822
26	3.157	2.841
27	3.178	2.859
28	3.199	2.876
29	3.218	2.893
30	3.236	2.908

All outliers (cells that marked in red in next paragraphs when representing the results) and the stragglers (marked in orange) that were defined based on the results of Grubbs analysis, were excluded from calculations of performance characteristics (final average numbers and the final reproducibility standard deviations among all laboratories).

Z-score

For the representation of consistency among all participating laboratories, the so-called Z score figures were used. The Z-scores were calculated accordingly to the formula:

Z-score = $(X_{measured} - X_{mean}) / SD$

Where	$X_{measured}$	Reported value, by each participating laboratory;
	X_{mean}	Mean value of all reported values (excluding straggles and outliers),
	SD	Reproducibility standard deviation.

Separately for each sample, the Z-score plots are given in Appendix A for the representation of the results on the total carbon content, and Appendix B when representing the results on the biogenic carbon content. In Appendices A and B, for each individual sample, the Z-score plots indicate how far is each laboratory from calculated average number.

5 Results

5.1 Pre-treatment of the samples

As it was already mentioned in the introduction, CEN TS 16640 specifies several possibilities for the conversion of the samples to CO_2-form ready for the 14C analysis. In this paragraph, the conversion that was done by each laboratory, is described.

Lab 1 and **Lab 7** used a calorimetric bomb for combustion of the samples. Where it was necessary, different catalysts to enhance the combustion were used (see further in the report for information for each sample).

In **Lab 2**, different subsamples were combusted to CO_2 and also measured on delta13C value with a combined Elementar Isotope Cube-Isoprime100 system (Isotope Ratio Mass Spectrometry, IRMS). The percentages of carbon and nitrogen were also (automatically) determined with this system. The obtained CO_2 of each sample was cryogenically trapped in a flask. Sample 6, biogas, was converted to CO_2 in a different combustion system that is described further in this report.

Lab 3 used a specific Macro-Element analyser to convert the samples into carbon dioxide, with subsequent with trapping and purifying of the CO_2.

In **Lab 4**, a tin capsule with a sample was placed in a nickel sleeve, injected into a high temperature furnace (975°C) and burnt in high purity oxygen under static conditions. The tin capsules used for the sample container allow an initial exothermic reaction to occur, raising the temperature of combustion to over 1800°C. A further dynamic burst of oxygen was added at the end of the combustion process, to ensure total combustion of all inorganic and organic substances. The resulting combustion products pass through specialised reagents to ensure full combustion of any methane produced and to remove halogens, sulphur and phosphorous. This process ultimately results in the production of CO_2 from the elemental carbon, H_2O from the hydrogen, and nitrogen (N_2) and N-oxides. The combustion gases are then passed, using helium as a carrier gas, through a tube packed with pure copper wire at 620°C, to remove excess oxygen and to reduce the N-oxides to elemental nitrogen. After this stage the gases enter a mixing chamber, to ensure a homogeneous mixture at constant temperature and pressure is delivered to the detectors. The mixture then passes through a series of high-precision thermal conductivity detectors, each containing a pair of thermal conductivity cells. Between the first two cells was a water trap, the differential signal between the cells is proportional to the water concentration, which is a function of the amount of hydrogen in the original sample. Between the next two cells was a carbon dioxide trap for measuring carbon.

Lab 5 followed EN 13137 for the combustion of the samples where the total carbon present in the undried sample is converted to carbon dioxide in an oxygen containing gas flow, free of carbon dioxide.

Lab 8 used equipment which consisted of a tube furnace and a purification line for the conversion of the samples into carbon dioxide.

Lab 9: liquid samples and emulsions (samples 1, 2, 3, 5, 8, 10 and 11) were converted to CO_2 using sealed tube combustion. The carbon dioxide was converted to graphite by reduction with hydrogen over iron catalyst. Samples 4, 7, 9 and 12 were converted to carbon dioxide by combustion in an elemental analyser. For sample 6 (bio-gas), a portion of sample gas was transferred into a quartz tube with CuO and Ag wire and combusted to produce CO_2. The carbon dioxide was converted to graphite by reduction with hydrogen over iron catalyst (remark by the laboratory: pressure gauge on sample gas bottle indicated low pressure, but more than sufficient gas was available for the measurement).
Lab 10 used an elemental analyser with combustion furnaces maintained at 1000° C for conversion of samples into carbon dioxide.
No information is available from the rest of participating laboratories.

Most samples were analysed by all laboratories in „as received“ conditions with no special preparations. Only for few samples the pre-treatment was done and is describes below:

SAMPLE 1

For Sample 1 (35% volatile), several laboratories did a special pre-treatment in order to avoid the loss of carbon that could be present in the volatile part and in order to facilitated the combustion of the sample.

Lab 1

Because of ignition and combustion difficulties, polyethylene bags with known carbon content (85.19%) and with known 14C content (3%) were used as combustion aids. The sample was combusted together with a bag and then the collected CO_2 gas was analysed on its 14C content. This resulted in 6% of biogenic carbon from collected CO_2. In turn, recalculated value for the true biogenic content of the sample itself equals 13%.

Lab 2

For the sample, the following analysis method has been applied by Lab 2: two subsamples of 4-10 mg each (based on estimated %C) were weighted in small tin capsules. As the sample was volatile, these subsamples were weighted in tin capsules with chromosorb material in order to absorb the materials and prevent leakage and loss of the material before combustion.

Lab 4

The elemental analysis and combustion experiments for the sample was performed on air-dried sample. Lab 4 found that combustion of the sample was not possible without the addition of benzoic acid. For 14C measurements, this obviously had an impact: the sample CO_2 is in fact 76.5% from benzoic acid and only 23.5% from the sample. The carbon content and the recovery values were corrected for this. The biogenic carbon fraction was found to be 3% of 14C when uncorrected and 13% after the corresponding correction on the carbon from benzoic acid.

The laboratory considered that for the samples presented as aqueous solutions it is of need to remove the water to get combustion to work, yet not evaporate any volatile components of each formulation. Therefore the sample was literately painted onto the inside of a glass vial and left the vial unsealed overnight. This was done for smaller and bigger subsamples. The data from the mass loss before and after evaporation were used to estimate the evaporated volatile part:

Sample (small subsample)	
Sample mass, g	1.32
Dry mass, g	0.90
Fraction dry mass	68%
Sample (bigger subsample)	
Sample mass, g	10.73
Dry mass, g	8.14
Fraction dry mass	76%

Lab 7

The sample was vacuum dried at 40C for 17 hours (solid after drying). On order to facilitate the combustion of the sample, the combustion enhancer $C_{16}H_{34}$ was used, with total carbon fraction of 85%. The biogenic carbon fraction was 3% as determined by an AMS for a pure enhancer.

The elemental analysis resulted in a carbon content of 136g of carbon per 1kg of dried material. The dry weight content was 66.5%, so after correction it should be 90g C/kg of wet sample.

Both wet sample and vacuum dried sample gave no combustion at 30bar oxygen environment using a bomb calorimeter. Combustion of the wet sample was only possible after adding drying material ($MgSO_4$) and a fire enhancer ($C_{16}H_{34}$) . The recalculated value for the true biogenic content of the sample itself was found to be 10%.

<u>SAMPLE 4</u>

Lab 9

A 2x2cm piece was cut from the corner of received sample and ground to coarse fragments/powder in IKA mill, and to finer powder in ball mill, then sieved at 425µm. The powder was used for combustion. Carbon dioxide was generated by elemental analyser combustion. Sample carbon dioxide was converted to graphite by reduction with hydrogen over iron catalyst.

<u>SAMPLE 5</u>

Lab 2

The same pre-treatment as for Sample 1.

<u>SAMPLE 6</u>

Lab 1

The installation and the procedure for the bio-bas combustion were described in Deliverable 3.4 of the KBBPPS.

Lab 2

The sample was converted to CO_2 in a combustion system as follows: the gas cylinder with biogas (the mixture of CO_2 and CH_4) was connected to a vacuum pumped combustion system which is developed by Lab 2 for this kind of application. Approximately 40 ml of gas was brought into this system. The gas was let into a system consisting a CuO-oven (heated at 850°C), a cryogenic H_2O trap (-78°C; ethanol/dry ice) and a volume with a magnetic stirrer that pushes the gas in the system from the volume behind the CuO-oven towards the volume before the CuO-oven with a certain frequency. The created flow in the system is used to force the CH_4 several times through the CuO-oven to obtain maximal combustion efficiency. After a certain time period, the formed CO_2 fraction in the gas sample was cryogenically trapped (-196°C; liquid N_2). The combustion of the CH_4 fraction was ended as soon as the pressure in the system did not drop any further (which indicates that no CO_2 is formed and trapped anymore). The remaining gas was pumped away and the trapped CO_2 fraction was let through a vacuum pumped Ag/Cu-oven (450°C) to remove any formed sulphur and nitrogen oxides before it was trapped in a second cryogenic CO_2 trap (-196°C; liquid N_2). Finally the CO_2 was put into two different 20-mL flasks for 13C (IRMS) and 14C analysis, respectively. The flask for 14C analysis contained Sulfix (WAKO, 8-20 mesh) to remove sulphur-containing components in the gas (which hamper a fast graphitisation of the CO_2). The CO_2 with Sulfix was heated for one night. The biogas sample has been combusted only once and the percentage carbon could not be determined with the used combustion system.

Lab 9

Remark by the laboratory: when the gaseous sample arrived, the pressure gauge was sitting on zero. It is possible there was a problem with the gauge, but it is also conceivable that some gas had leaked. The gas remaining inside the cylinder was almost entirely carbon containing, and there was no gas that would not freeze into liquid nitrogen (i.e. no air) so if there was any leakage into the cylinder during shipping it must have been small. A portion of sample gas was transferred into a quartz tube with CuO and Ag wire and was combusted in order to produce CO_2.

SAMPLE 7

Lab 9

Description of sample when received: plastic jar with small spherical off white plastic granules. Sub sample was taken out; approximately 20 mg was needed to be ground up for combustion. Pre-treatment description: beads were crushed up to coarse powder. Carbon dioxide was generated by elemental analyser combustion and 0.8mgC was obtained.

SAMPLE 8

Lab 1

Because of ignition and combustion difficulties, polyethylene bags with known carbon content (85.19%) and with known 14C content (3%) were used as combustion aids.

The sample was combusted together with a bag and then the collected CO_2 gas was analysed on its 14C content. This resulted in 37% of biogenic carbon from collected CO_2. In turn, recalculated value for the true biogenic content of the sample itself equals 94%.

Lab 4

The same as for Sample 1.

Lab 7

The sample was vacuum dried at 40C for 17 hours (solid after drying). On order to facilitate the combustion of the sample, the combustion enhancer $C_{16}H_{34}$ was used, with total carbon fraction of 85%. The biogenic carbon fraction was 3% as determined by an AMS for a pure enhancer. Both wet sample and vacuum dried sample gave no combustion at 30bar oxygen environment using a bomb calorimeter. Combustion of the wet sample was only possible after adding drying material ($MgSO_4$) and a fire enhancer (hexadecane). The biogenic carbon content of the sample itself was the recalculated to be 98%.

SAMPLE 10

Lab 1

Because of ignition and combustion difficulties, polyethylene bags with known carbon content (85.19%) and with known 14C content (3%) were used as combustion aids. The sample was combusted together with a bag and then the collected CO_2 gas was analyzed on its 14C content. This resulted in 28% of biogenic carbon from collected CO_2. In turn, recalculated value for the true biogenic content of the sample itself equals 72%.

Lab 4

The same as for Sample 1.

Lab 7

The sample was vacuum dried at 40C for 17 hours (solid after drying). On order to facilitate the combustion of the sample, the combustion enhancer $C_{16}H_{34}$ was used, with total carbon fraction of 85%. The biogenic carbon fraction was 3% as determined by an AMS for a pure enhancer. Both wet sample and vacuum dried sample gave no combustion at 30bar oxygen environment using a bomb calorimeter. Combustion of the wet sample was only possible after adding drying material ($MgSO_4$) and a fire enhancer (hexadecane). The biogenic carbon content of the sample itself was the recalculated to be 71%.

SAMPLE 11

Lab 1

Because of ignition and combustion difficulties, polyethylene bags with known carbon content (85.19%) and with known 14C content (3%) were used as combustion aids. The sample was combusted together with a bag and then the collected CO_2 gas was analysed on its 14C content. This resulted in 59% of biogenic carbon from collected

CO_2. In turn, recalculated value for the true biogenic content of the sample itself equals 92%.

Lab 4

The same as for Sample 1.

Lab 7

The sample was vacuum dried at 40C for 17 hours (solid after drying). On order to facilitate the combustion of the sample, the combustion enhancer $C_{16}H_{34}$ was used, with total carbon fraction of 85%. The biogenic carbon fraction was 3% as determined by an AMS for a pure enhancer. Both wet sample and vacuum dried sample gave no combustion at 30bar oxygen environment using a bomb calorimeter. Combustion of the wet sample was only possible after adding drying material ($MgSO_4$) and a fire enhancer (hexadecane). The biogenic carbon content of the sample itself was the recalculated to be 98%.

5.2 Results on the total carbon content

The total carbon content as presented in Table 2, was measured using an elemental analyser. Red cells indicate an outlier (based on the Grubbs test). Orange cells indicate a straggler (based on the Grubbs test). Grey cells indicate that no measurement on that sample was performed. The column “Supplier” represents data provided by the suppliers of the samples.

Table 2. Total carbon content

	Total C fraction, %										
	Supplier	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10
SAMPLE 1	11.5	10.5	10.4	10.4	13.9	9.4		9.0		10.8	10.5
SAMPLE 2	45±5	42.6	46.0	46.4	46.2	45.7		46.7		45.3	45.4
SAMPLE 3	45±5	42.4	42.1	42.8	42.1	42.7		42.3		44.2	42.7
SAMPLE 4	-	42.9	42.5	40.5	42.4	42.0		45.4		42.6	45.3
SAMPLE 5	84.6	84.6	84.1	83.5	83.5	84.6				81.3	84.6
SAMPLE 6	-										
SAMPLE 7	77.4	77.8	76.8	77.8	76.6	76.9	70.8	55.9		76.1	76.3
SAMPLE 8	15.4	15.4	17.9	15.5	28.4	15.7		13.7		17.3	15.7
SAMPLE 9	69.5	64.2	63.5	66.0	64.6	63.3	47.0	68.0		64.2	68.1
SAMPLE 10	12.4	13.7	13.2	13.2	21.3	13.0		12.9		14.7	13.5
SAMPLE 11	39.6	39.9	38.8	39.3	62.5	39.8		34.4		43.7	40.2
SAMPLE 12	49.3	45.3	45.8	44.8	46.5	45.7	41.3	46.0		46.0	49.4

Performance characteristics

Table 3 below presents the performance characteristics that are obtained based on the results of the measurements given in Table 2. For each sample, the performance characteristics include the total number of participating laboratories, the number of outliers and/or stragglers, the percentage of the outlying values with respect to the total number of measurements, the overall average and the reproducibility standard deviations (S_R). For every sample, the overall average is calculated as the mean value of all reported measured values excluding the numbers that based on the results of the Grubbs test were regarded as outliers and/or stragglers. Subsequently, the same set of reported measured values was taken for the calculations of the reproducibility standard deviation (indicates the deviation among the laboratories with respect to the calculated average value). The coefficient of the variation of the reproducibility (CV_R) is also presented. Typically the CV_R is calculated as a ratio between the S_R and the overall average. In this content, for a given sample, lower CV_R means less variation is present, indicating that the reproducibility is higher.

Table 3. Performance characteristics based on the results of round robin test for total carbon content in each sample. S_R is the reproducibility standard deviation, CV_R is the coefficient of the variation of the reproducibility.

SAMPLE	No .of laboratories	No. of outliers and stragglers	No. of outlier and straggler free	% of outlying values	Total C, overall average, %	S_R, %	CV_R, %
SAMPLE 1	8	1	7	12.5	10.1	0.7	6.9
SAMPLE 2	8	1	7	12.5	46.0	0.5	1.1
SAMPLE 3	8	1	7	12.5	42.4	0.3	0.7
SAMPLE 4	8	0	8	0.0	42.9	1.7	3.9
SAMPLE 5	7	1	6	14.3	84.1	0.5	0.6
SAMPLE 7	9	2	7	22.2	76.9	0.7	0.9
SAMPLE 8	8	1	7	12.5	15.9	1.4	8.8
SAMPLE 9	9	1	8	11.1	65.2	1.9	2.9
SAMPLE 10	8	2	6	25.0	13.3	0.3	2.3
SAMPLE 11	8	1	7	12.5	39.5	2.7	6.8
SAMPLE 12	9	0	9	0.0	45.6	2.1	4.6

As it can be seen from the calculated performance characteristics, the highest coefficients of the variation of the reproducibility are observed for Samples 8, 1 and 11 (correspondingly 8.8%, 6.9% and 6.8%). This can be explained by the fact that these samples were relatively „difficult" to combust: Samples 8 and 11 contained large fraction of water; Sample 1 contained 35% of volatile component and very small amount of carbon.

Detailed representation of the results on the total carbon content for each sample individually including measured average, reproducibility standard deviation, min and max values, is given in Appendix A.

5.3 Results on the biogenic carbon content

The data below represent the results of the 14C measurements done by AMS and LSC laboratories.

Red cells indicate an outlier (based on the Grubbs test). Orange cells indicate a straggler (based on the Grubbs test). Grey cells indicate that no measurement on that sample was performed. The column “Supplier” represents data provided by the suppliers of the samples.

Table 4. Biogenic carbon content

	Biogenic carbon fraction, %											
	Supplier	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab 10	Lab 11
SAMPLE 1		13	9	8	13	40		10	20	10	10	9
SAMPLE 2		16	13	13	14	22		14	18	13	15	14
SAMPLE 3		97	96	95	97	31		97	97	96	98	97
SAMPLE 4		95	95	94	94	29		92	95	93	93	95
SAMPLE 5		99	99	95	99	77		99	95	94	99	100
SAMPLE 6		95	95			0				98		
SAMPLE 7	100	99	98	96	98	99	99	98		97	98	98
SAMPLE 8	97	94	82	93	96	95		98	94	94	95	96
SAMPLE 9	14	14	11	10	13	11	12	13	25	12	13	13
SAMPLE 10	81	72	73	71	74	91		71	78	73	74	73
SAMPLE 11	99	92	93	93	86	95		98	95	93	93	95
SAMPLE 12	92	99	100	99	100	100	100	99	100	98	100	98

Lab 3, Lab 8 and Lab 10 did the 14C analysis using the LSC technique, while the results reported by the rest of the laboratories are obtained by performing an AMS analysis on each sample. As can be seen from Table 4, the results obtained by these two different techniques are equivalent.

NOTE: Lab 5 analyzed Samples 1-6 using an LSC, while Samples 7-12 were analyzed using an AMS. All LSC results performed by Lab 5 were regarded as outliers based on the Grubbs analysis and were excluded from further considerations. After communication with Lab 5, this mismatch in the results can be related to the incorrect use of the LSC technique or to improper combustion of the samples. The result of the 14C analysis of Lab 2 on Sample 8, of Lab 4 on Sample 11 and of Lab 8 on Samples 1 and 9 were regarded as stragglers or outliers. However, it can be considered as random deviation more than a systematic error, since the results on the rest of the samples reported by these laboratories are consistent with the rest of laboratories.

Performance characteristics

Similarly to the results on the total carbon content, the performance characteristics of the bio-based carbon determination include the total number of participating laboratories, the number of outliers and/or stragglers, the percentage of the outlying values with respect to the total number of measurements, the overall average and the reproducibility standard deviations (S_R). For every sample, the overall average is calculated as the mean value of all reported measured values excluding the numbers that based on the results of the Grubbs test were regarded as outliers and/or stragglers. Subsequently, the same set of reported measured values was taken for the calculations of the reproducibility standard deviation (indicates the deviation among the laboratories with respect to the calculated average value). The coefficient of the variation of the reproducibility (CV_R) is also presented. Typically the CV_R is calculated as a ratio between the S_R and the overall average. For a given sample, lower CV_R means less variation is present, indicating that the reproducibility is higher.

Table 5. Performance characteristics based on the results of round robin test for biogenic carbon content in each sample. S_R is the reproducibility standard deviation, CV_R is the coefficient of the variation of the reproducibility.

SAMPLE	No of laboratories	No of outliers and stragglers	No of outlier and straggler free	% of outlying values	The overall average, % 14C	S_R, % 14C	CV_R, %
SAMPLE 1	10	2	8	20	10.2	1.8	17.7
SAMPLE 2	10	1	9	10	14.4	1.5	10.4
SAMPLE 3	10	1	9	10	96.7	0.8	0.8
SAMPLE 4	10	1	9	10	94.0	1.4	1.5
SAMPLE 5	10	1	9	10	97.3	2.3	2.4
SAMPLE 6	4	1	3	25	96.0	1.7	1.8
SAMPLE 7	10	0	10	0	98.0	1.0	1.0
SAMPLE 8	10	1	9	10	95.0	1.4	1.5
SAMPLE 9	11	1	10	9	12.2	1.2	9.8
SAMPLE 10	10	1	9	10	73.2	2.0	2.7
SAMPLE 11	10	1	9	10	94.1	1.8	1.9
SAMPLE 12	11	0	11	0.0	99.3	0.8	0.8

As it can be seen from the calculated performance characteristics, the highest coefficients of the variation of the reproducibility are observed for Samples 1, 2 and 9 (correspondingly 17.7%, 10.4% and 9.8%).

For Sample 1, this can be explained by difficulties that laboratories met with achieving the complete combustion of the samples and with the possible loss of carbon that could be present in the volatile part of the sample. In case of Sample 9 (multilayer packaging film, consisting of parts of different colours with 1-2% difference in their carbon content), somewhat lower reproducibility can be related to the preparation of the representative sample (having a sample including all colours or burning the sample as a whole). For emulsion-like types of samples (f.e. Sample 2,) a homogeneity of such samples has to be ensured. This could explain the somewhat higher variation of the reproducibility among participating laboratories.

Further in Appendix B of this report, the results on the 14C content are presented for each sample individually, including measured average, reproducibility standard deviation, min and max values.

6 Conclusions

This report presents the results of the round robin assessment that was organised to investigate the performance characteristics of the method described in CEN TS 16640 for the bio-based carbon content determination, in order to convert the available technical specification into the European standard. The round robin assessment was initiated in the frameworks of the European Open-Bio project (www.biobasedeconomy.eu).

Statistical evaluations of the results was done by performing Grubbs test for the results on each sample reported by each laboratory. Outliers and stragglers that were defined based on the results of Grubbs analysis, were excluded from calculations of measured average numbers and the reproducibility standard deviations among all laboratories.

The results of performed assessment show a good consistency. The maximum number of outliers/stragglers (1 outlier, 1 straggler) when analysing the reported results on the 14C content, was observed for Sample 1 that was a paint with low carbon content and with a high volatile fraction (approximately 35%). This can be related to the combustion difficulties and possible loss of carbon that could be present in the volatile fraction. The maximum value for the variation of the coefficient of the reproducibility (17.7%) for the biogenic carbon content was observed for the same Sample 1 (10.2 ± 1.8 % of 14C as fraction of total carbon, see Table 5), that was one of the most challenging samples. Analyzing the calculated performance characteristics for the total carbon content, one can observe that the highest value for the variation of the reproducibility standard deviation for total carbon content was 8.8% (15.9 ± 1.47) for Sample 8 that was cosmetic emulsion with high water content (see Table 3). Relatively high variation in the coefficient of the reproducibility for Sample 1 and Sample 8 can be caused by combustion difficulties of these two samples: some laboratories used combustion enhancers and some did not. This can explain somewhat high values for the reproducibility variations, but despite differences in pre-treatment the calculated mean values for these samples were close to the ones reported by the suppliers of these samples. Neverheless, this has to be taken into account when converting paint-like and water containing samples into carbon dioxide.

Due to technical difficulties, only 4 of 11 laboratories were able to analyse the bio-gas sample (sample 6). Deliverable 3.4 of KBBPPS gives a description of an installation that can be used for the conversion of gaseous samples into the CO_2 form. If necessary this experience can be used, provided that all safety measures are ensured.

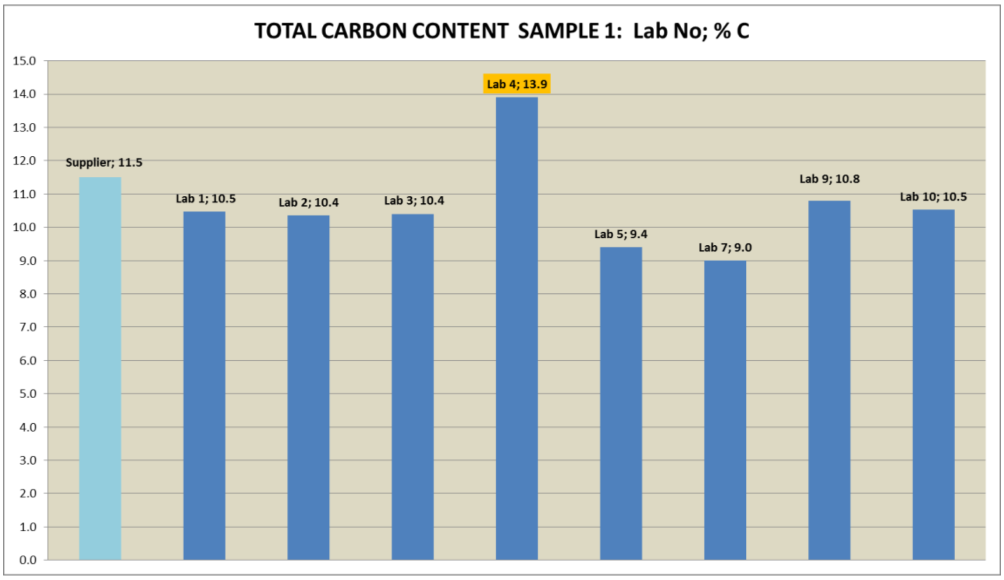
For the C14 analysis, the known LSC (Liquid Scintillation Counting) or the AMS (Accelerated Mass Spectrometry) techniques were used in this round robin assessment. 3 of 11 laboratories did the 14C analysis using the LSC method (no direct LSC was performed on any samples). By 8 laboratories the AMS analysis was used in order to determine the 14C amount in the delivered samples. The results of the round robin assessment indicates that no inconsistencies are observed for the results of the measurements when using AMS (Accelerated Mass Spectrometry) and LSC (Liquid Scintillation Counting) techniques and thus proves the equivalence of these two techniques.

Appendix A: Total carbon content and Z-scores for Samples 1-12

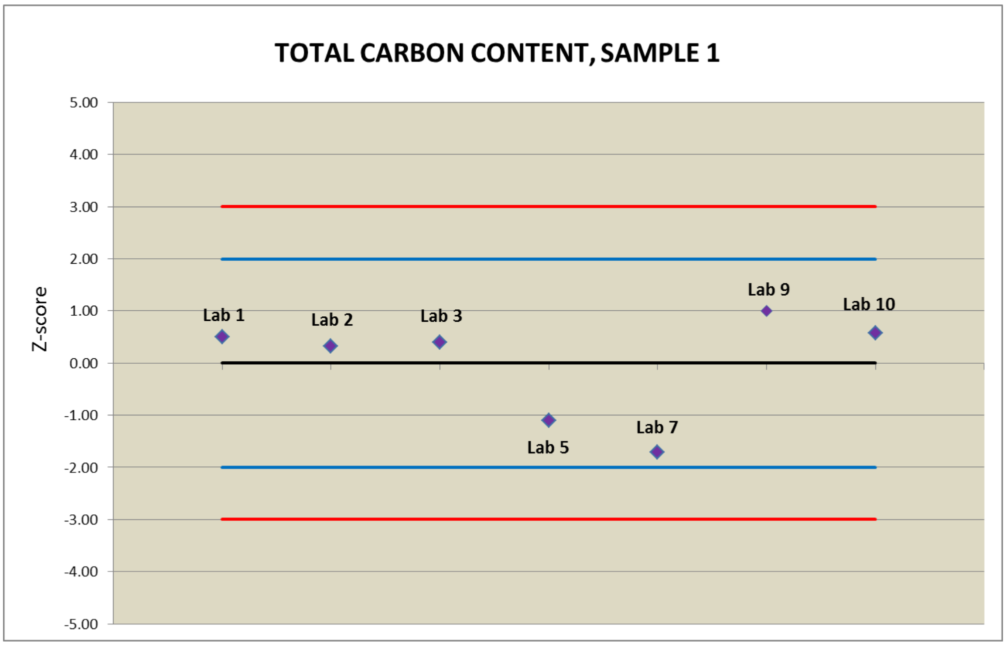
Below the results of the measurements of the total carbon content are presented separately for each of the 12 Samples. For each sample, the bar-plots give a comparison of the total carbon content reported by all participating laboratories. Outliers and stragglers are included in these plots and are marked orange for the stragglers and red for the outliers. The data from product suppliers are included as well.

Next, Z-score plots are presented separately for each sample (for the calculations of Z-scores see paragraph 4). Outliers and stragglers were excluded when calculating the average numbers and the Z-scores. In this representation, for each individual sample, the Z-score plots indicate how far each laboratory is from the calculated average number, which is depicted by the black line in the Z-score plots. Blue and red lines in the Z-score plots correspondingly indicate $2 \cdot S_R$ and $3 \cdot S_R$ borders, where S_R is the reproducibility standard deviation.

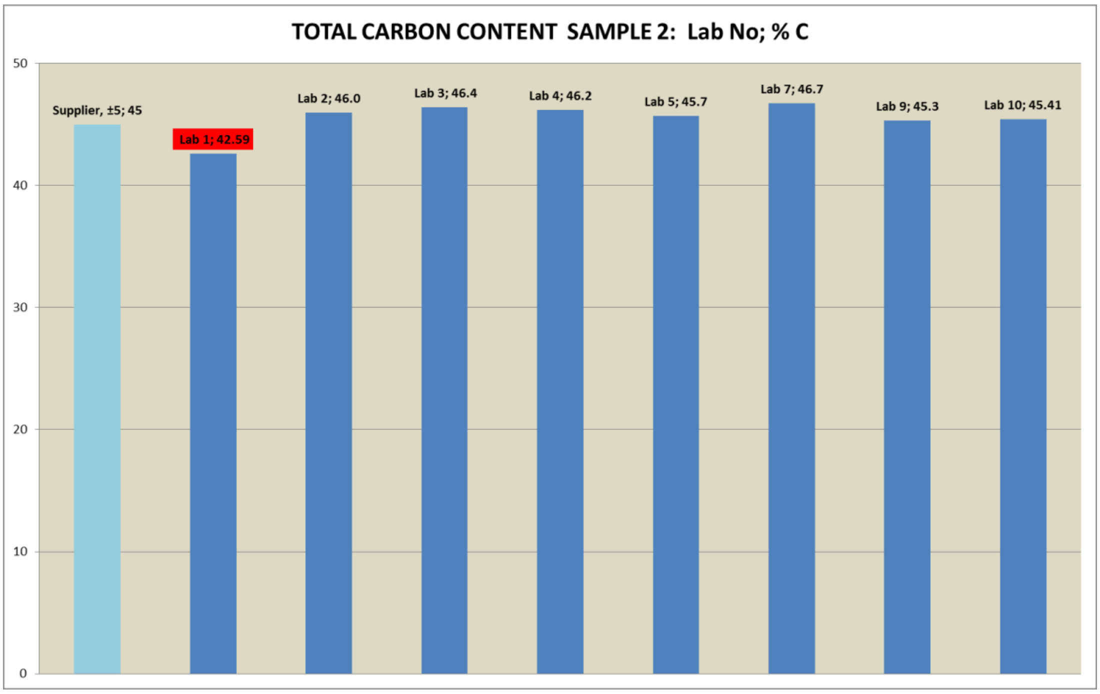
SAMPLE 1: White water soluble matt paint



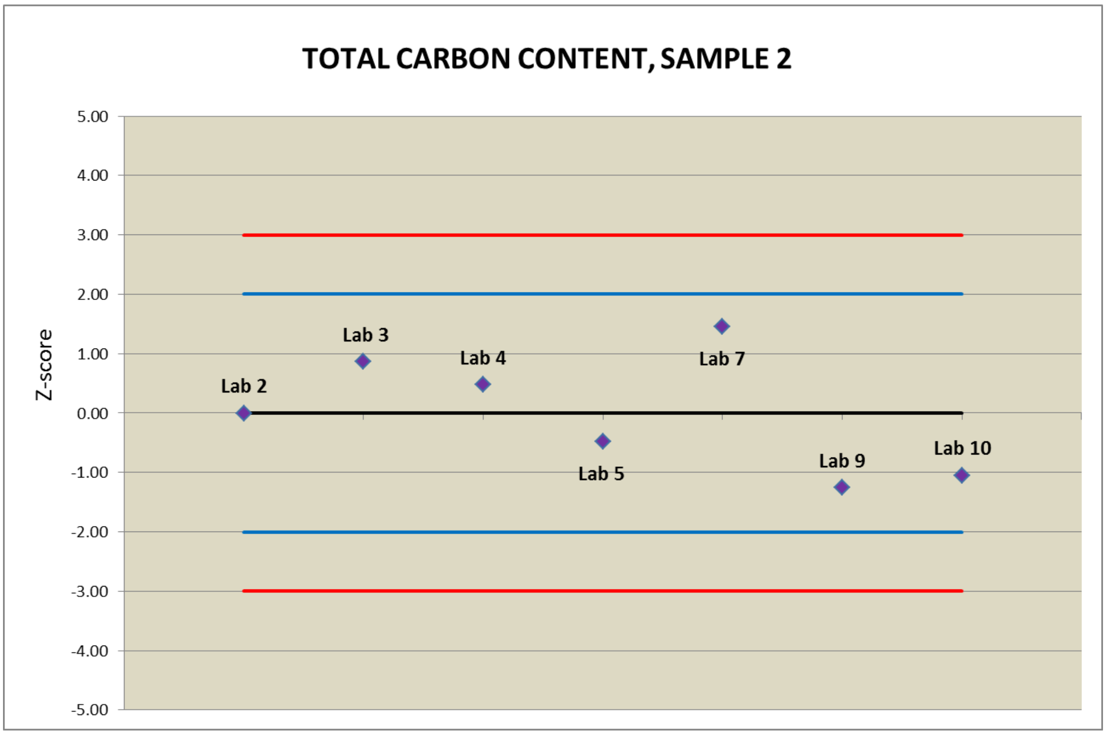
AVERAGE	10.1
STD	0.7
Min	9.0
Max	10.8



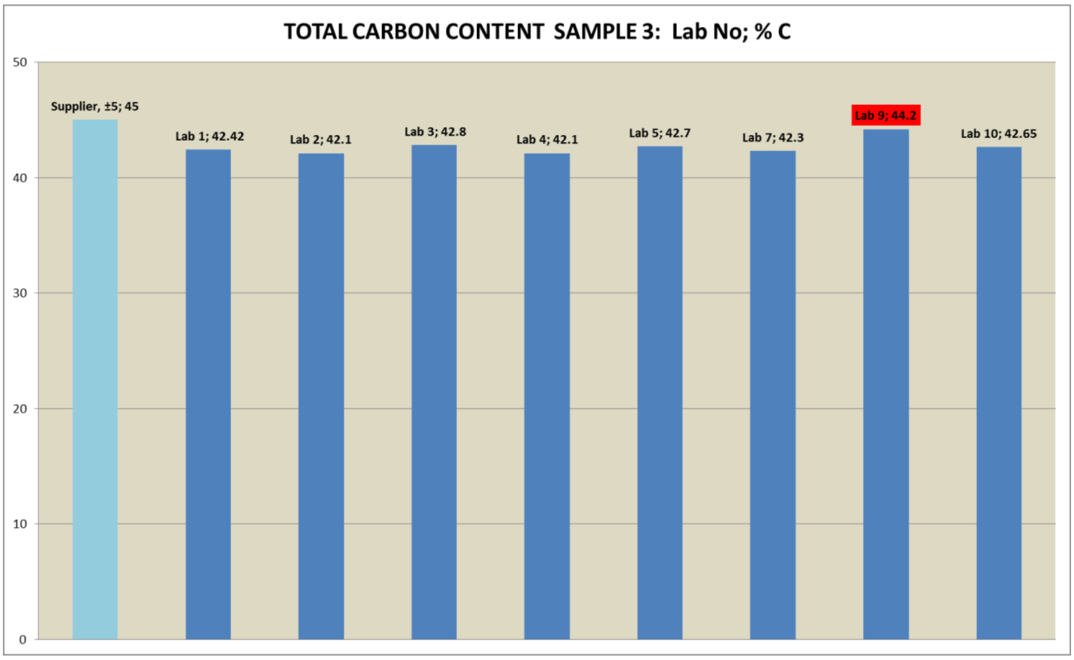
SAMPLE 2: White emulsion



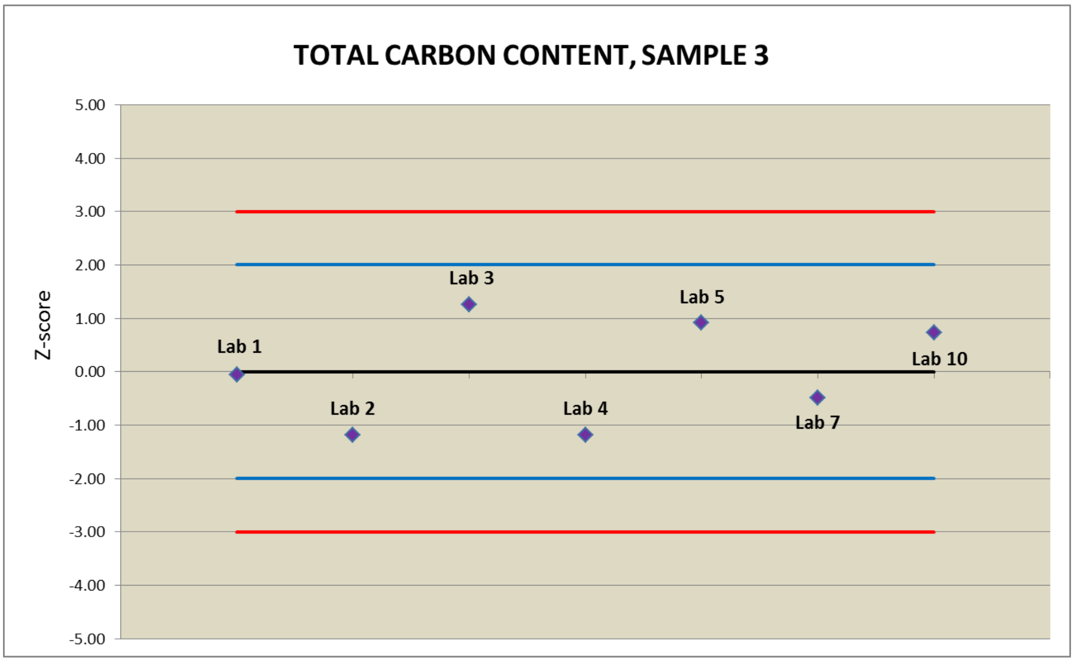
AVERAGE	46.0
STD	0.5
Min	45.3
Max	46.7



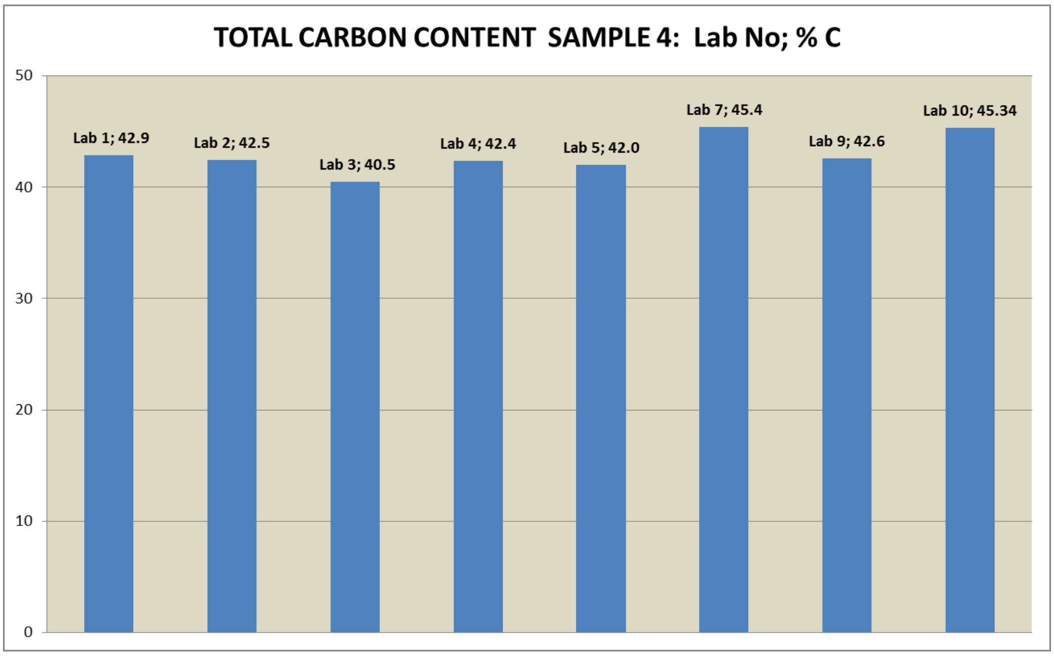
SAMPLE 3: White emulsion



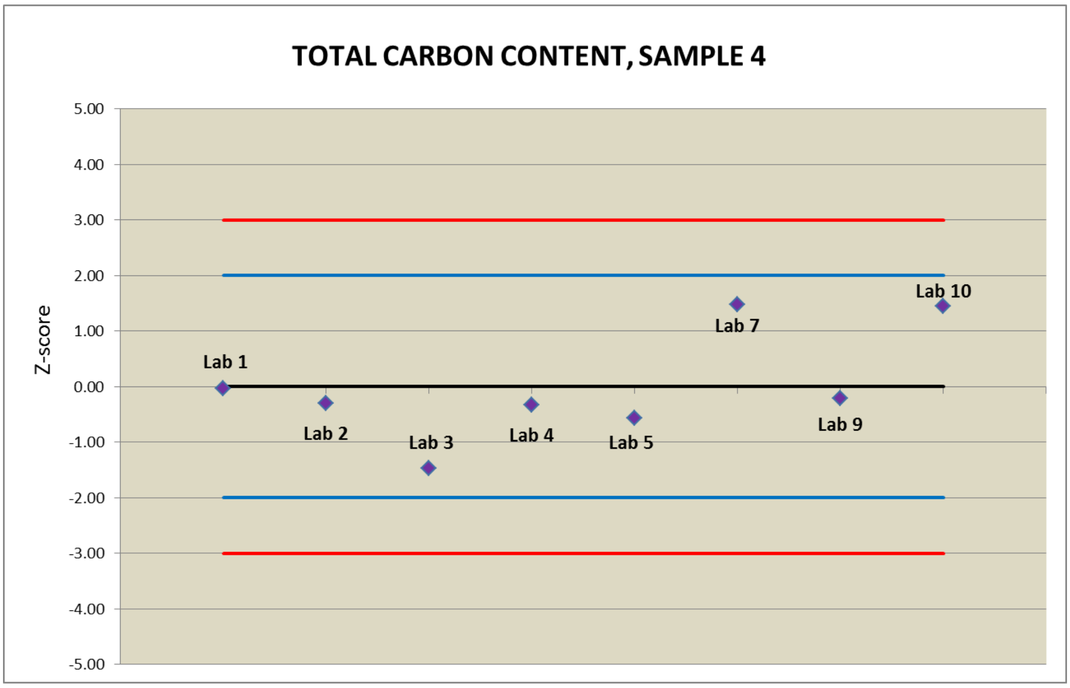
AVERAGE	42.4
STD	0.3
Min	42.1
Max	42.8



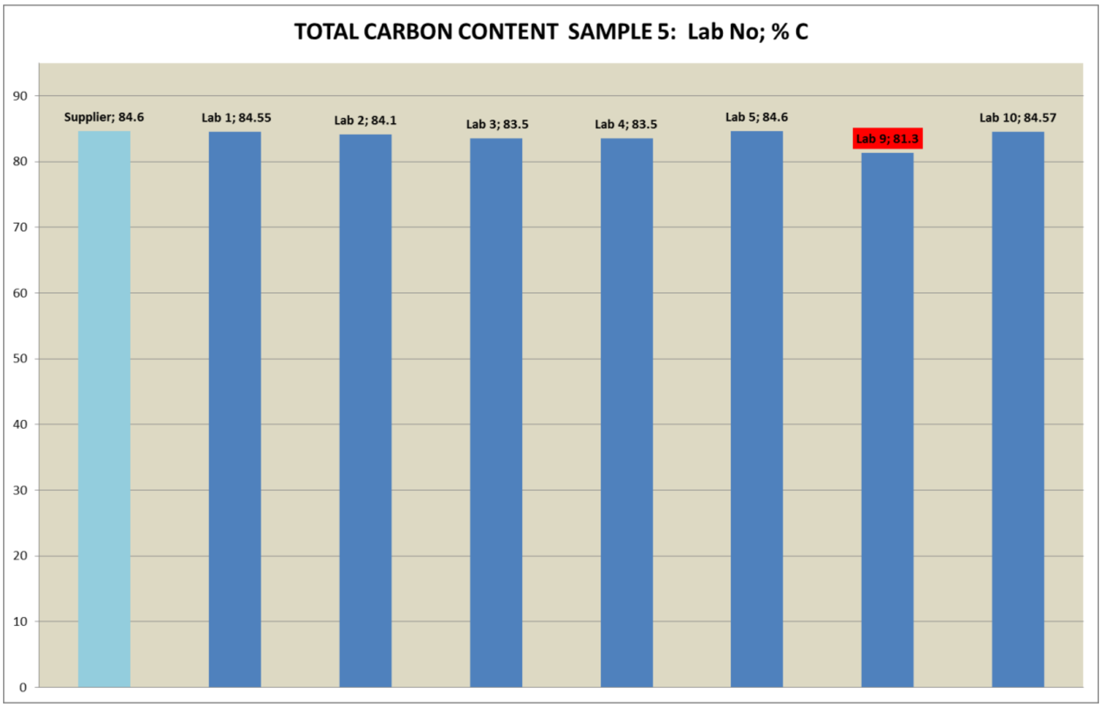
SAMPLE 4: Wheat straw panel



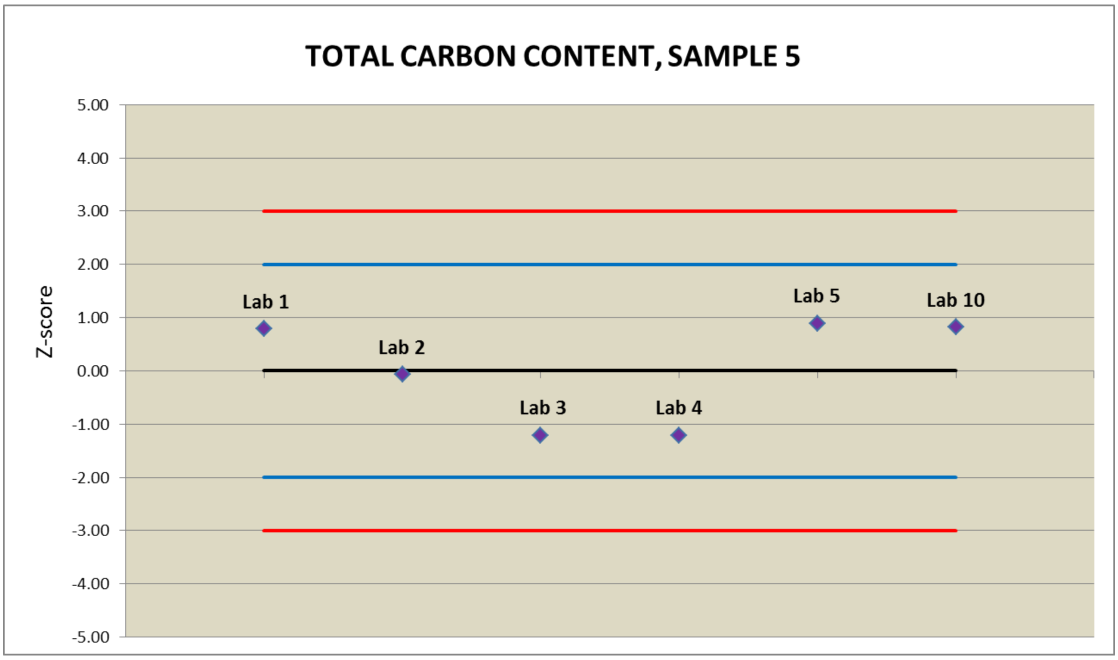
AVERAGE	42.9
STD	1.7
Min	40.5
Max	45.4



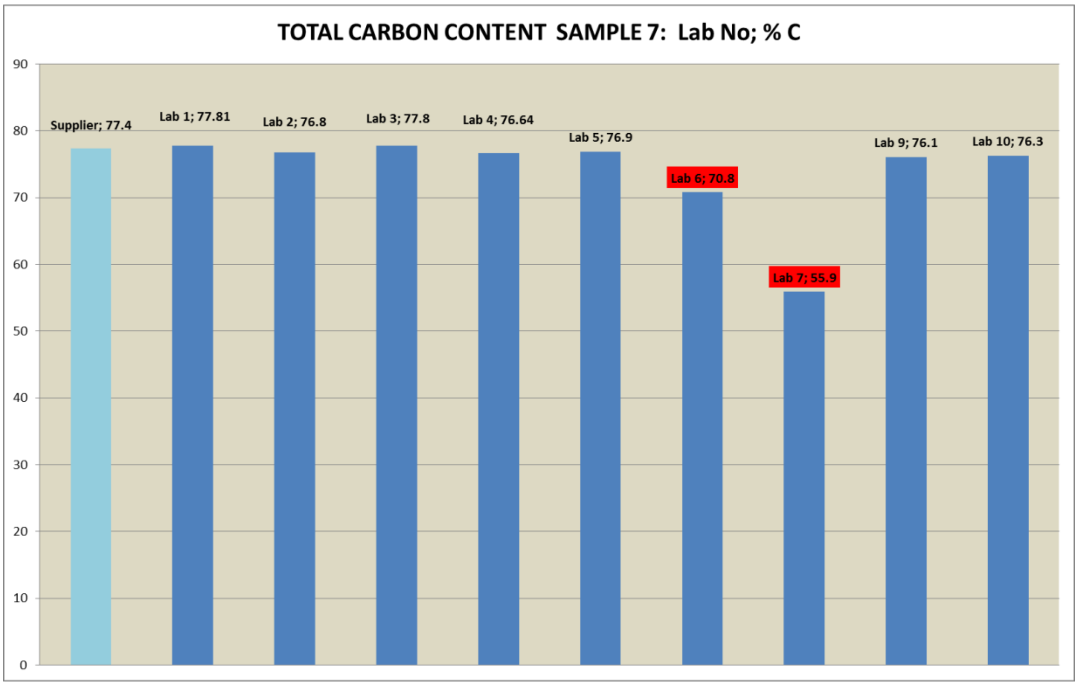
SAMPLE 5: Biodiesel



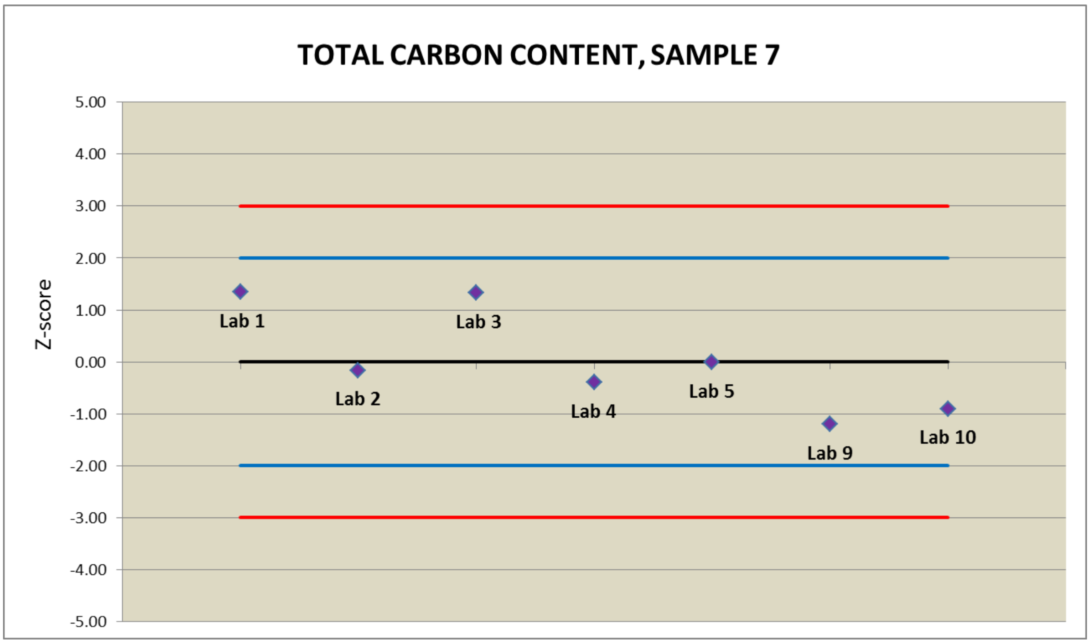
AVERAGE	84.1
STD	0.5
Min	83.5
Max	84.6



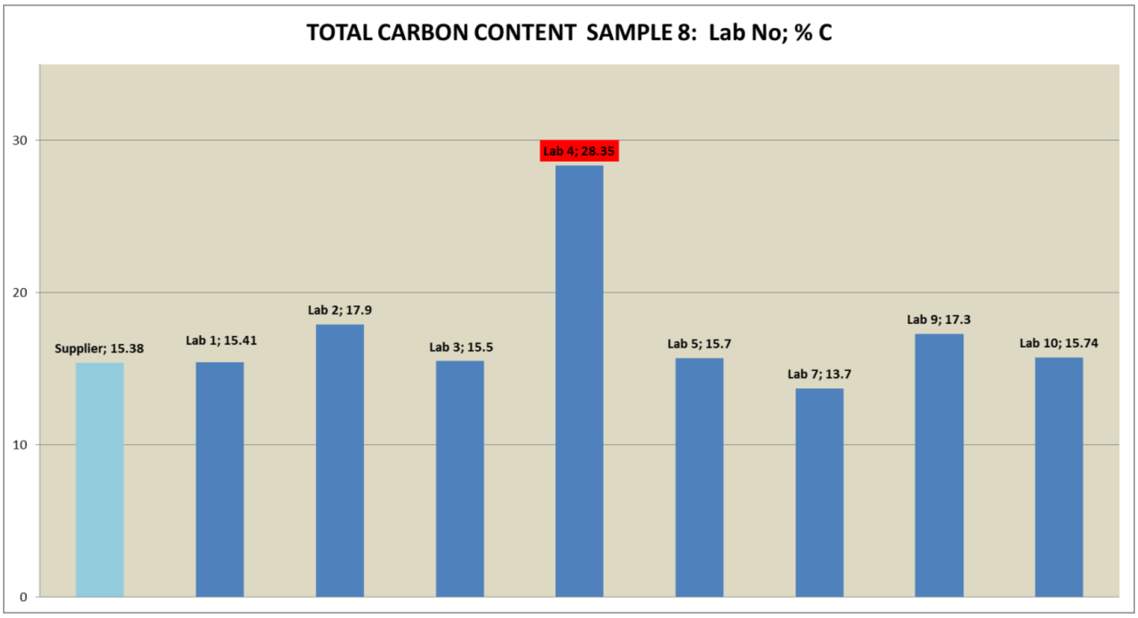
SAMPLE 7: White surfactant granules



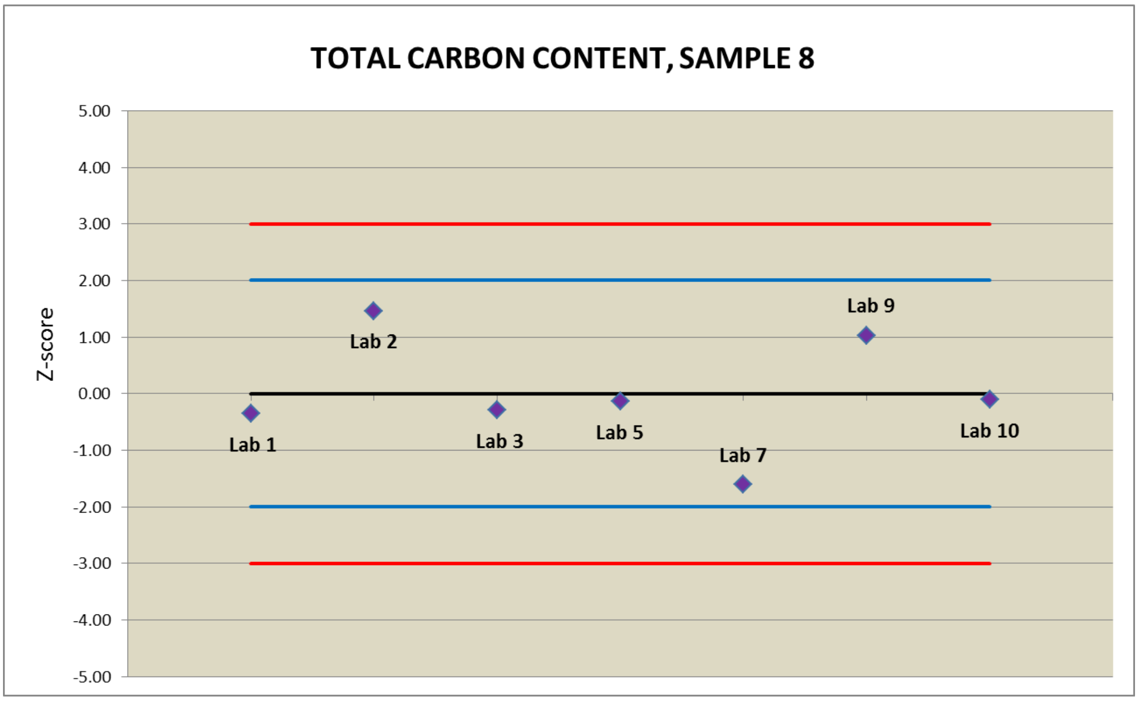
AVERAGE	76.9
STD	0.7
Min	76.1
Max	77.8



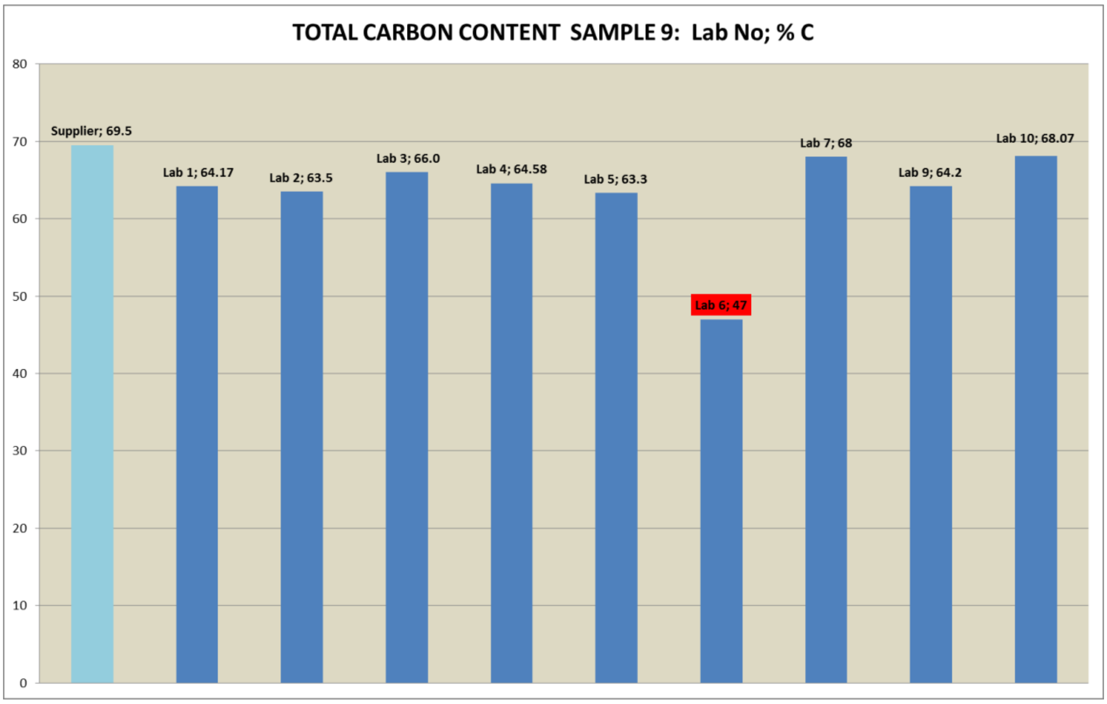
SAMPLE 8: Cosmetic emulsion



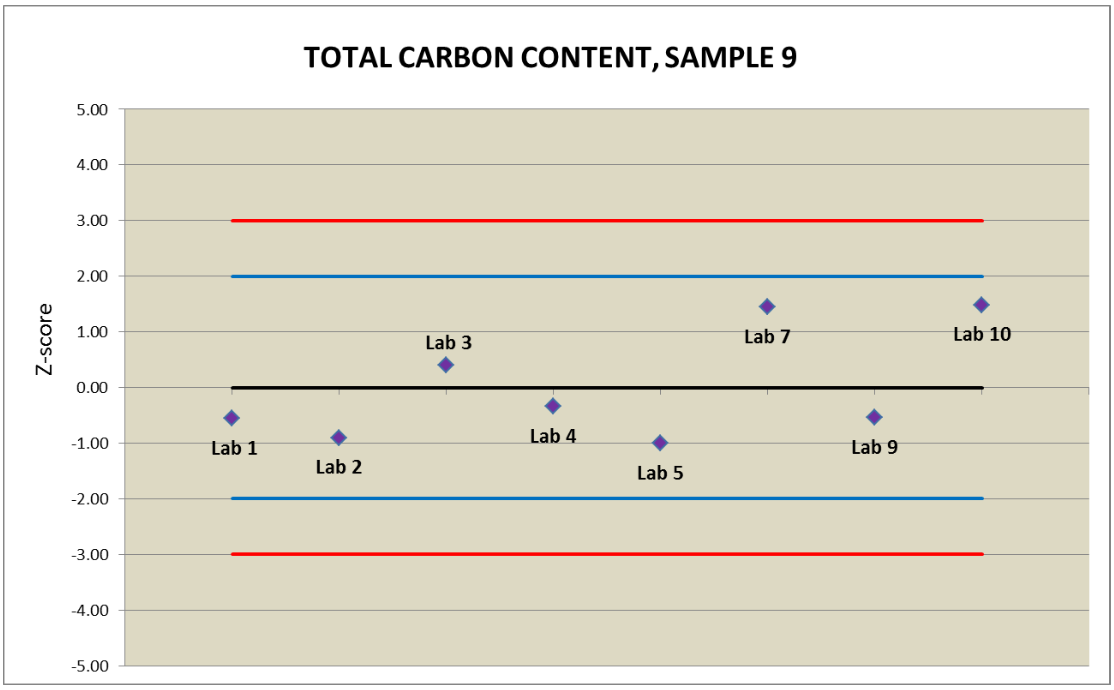
AVERAGE	15.9
STD	1.4
Min	13.7
Max	17.9



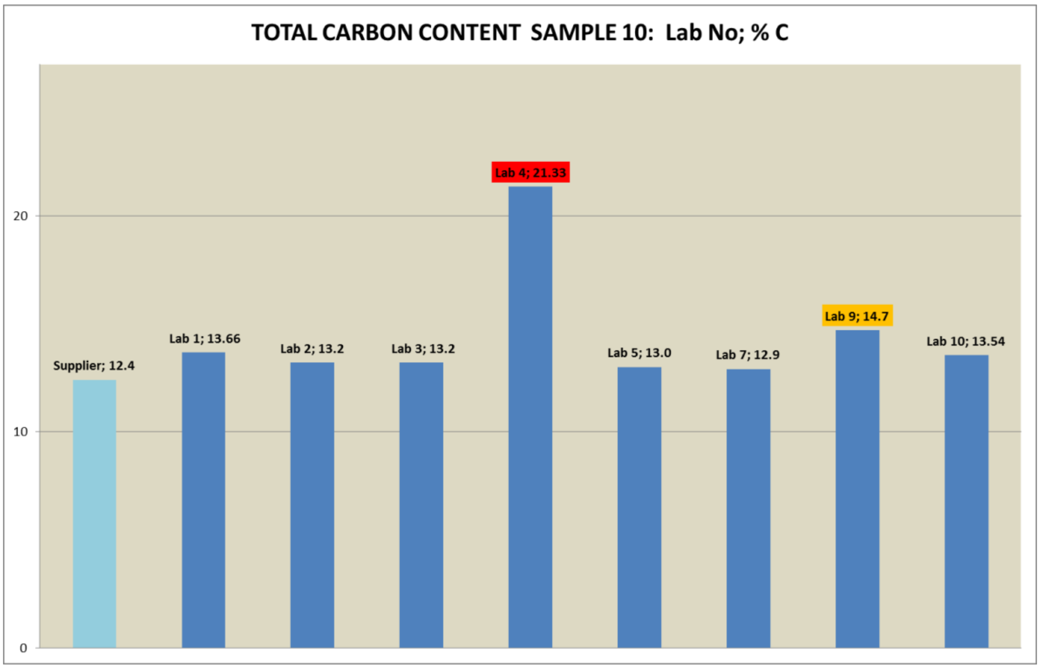
SAMPLE 9: Multilayer packaging film



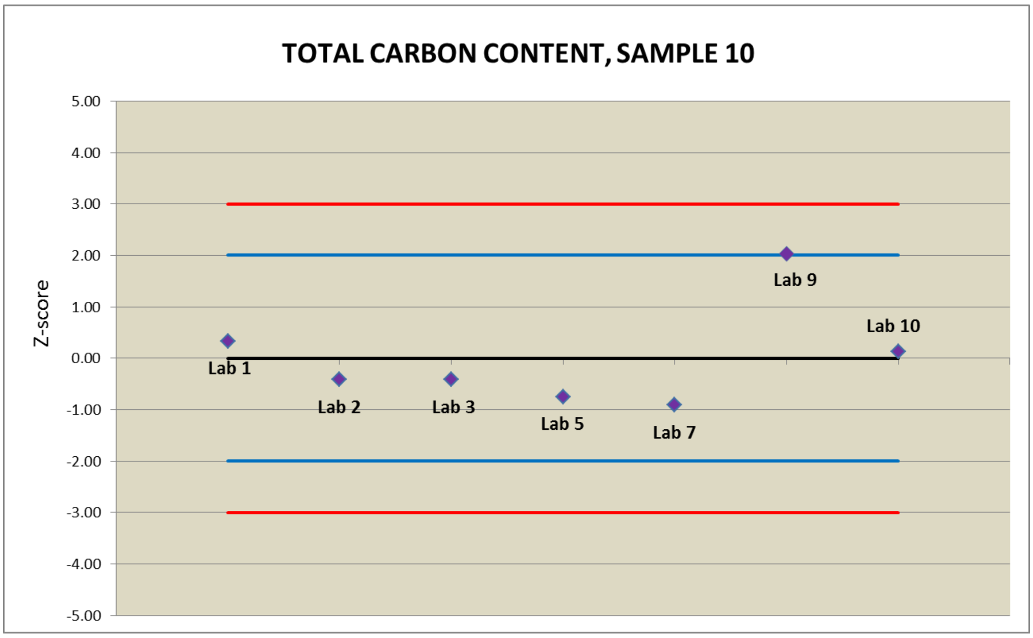
AVERAGE	65.2
STD	1.9
Min	63.3
Max	68.1



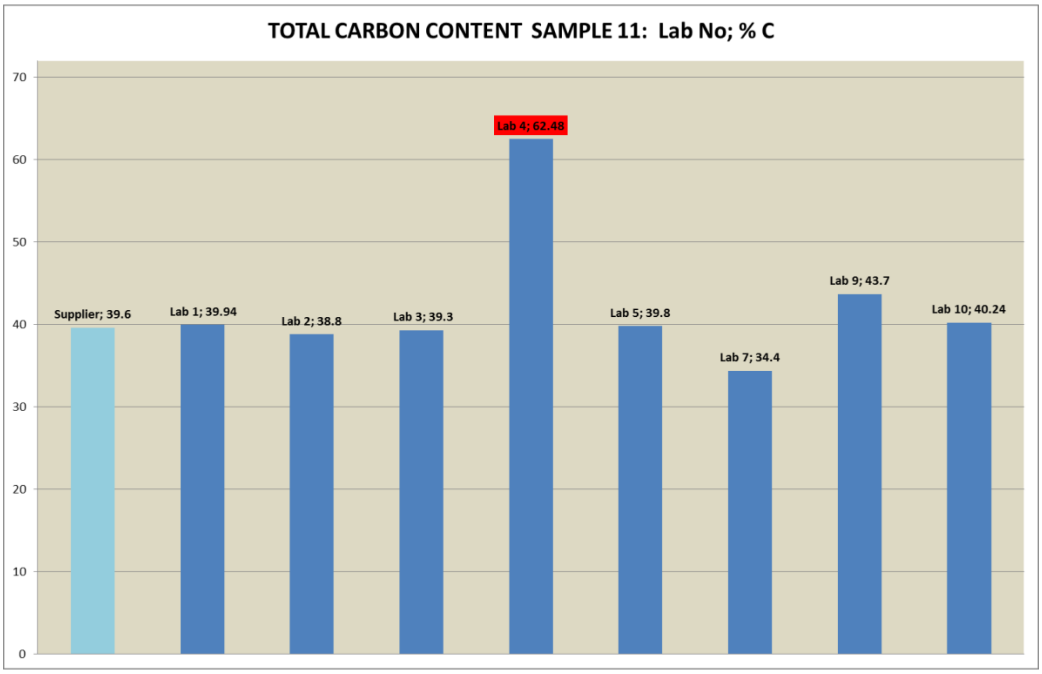
SAMPLE 10: Silk paint



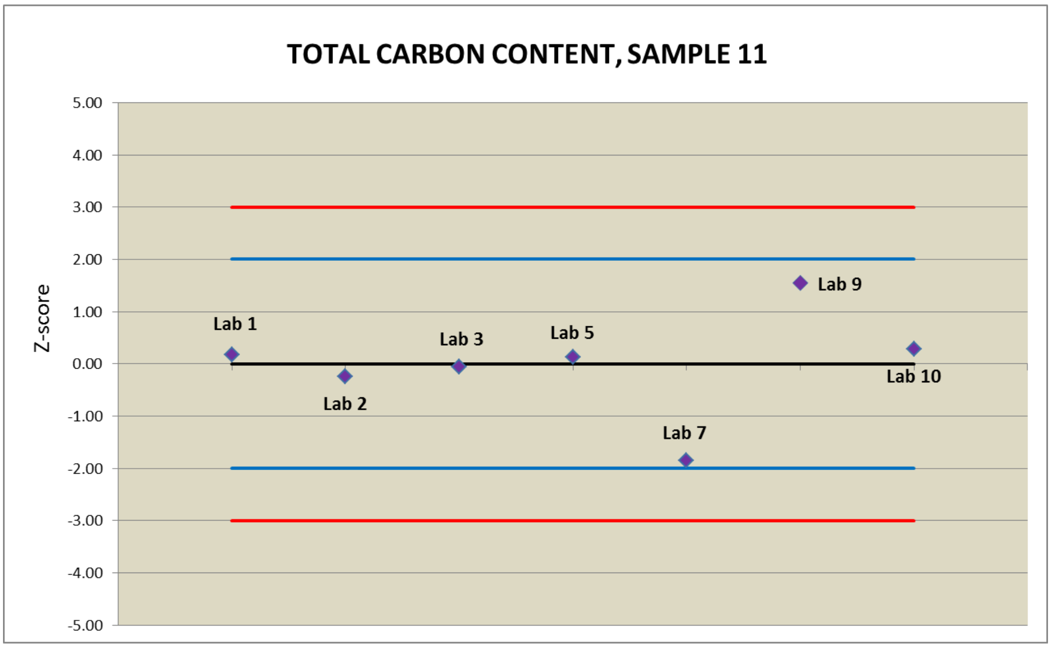
AVERAGE	13.5
STD	0.6
Min	12.9
Max	14.7



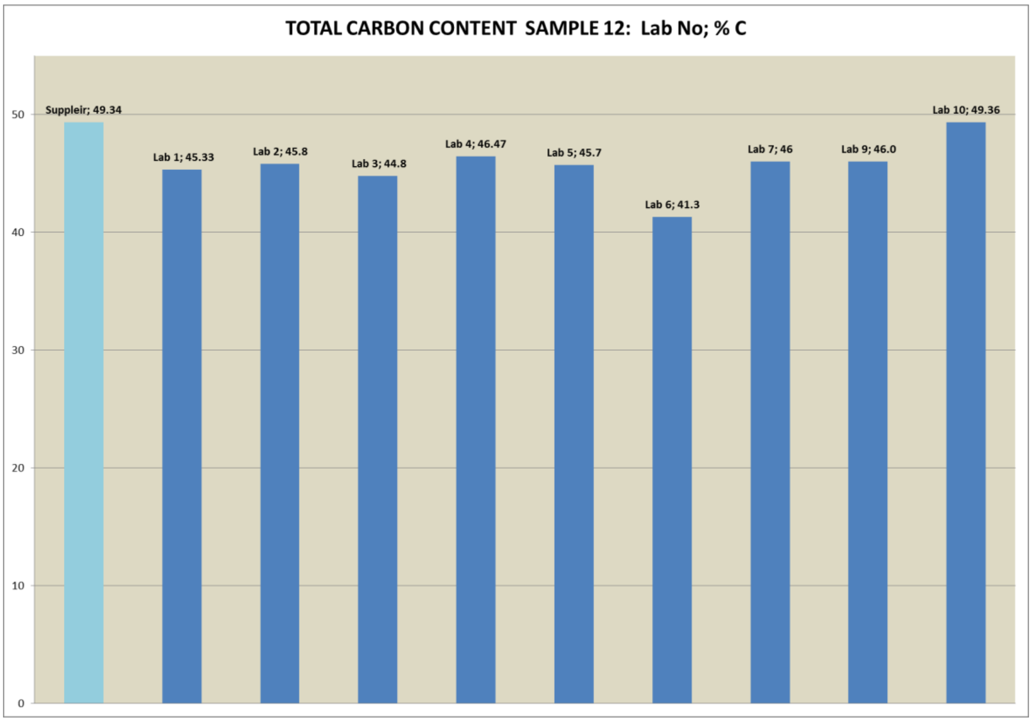
SAMPLE 11: Bio-based binder for paint



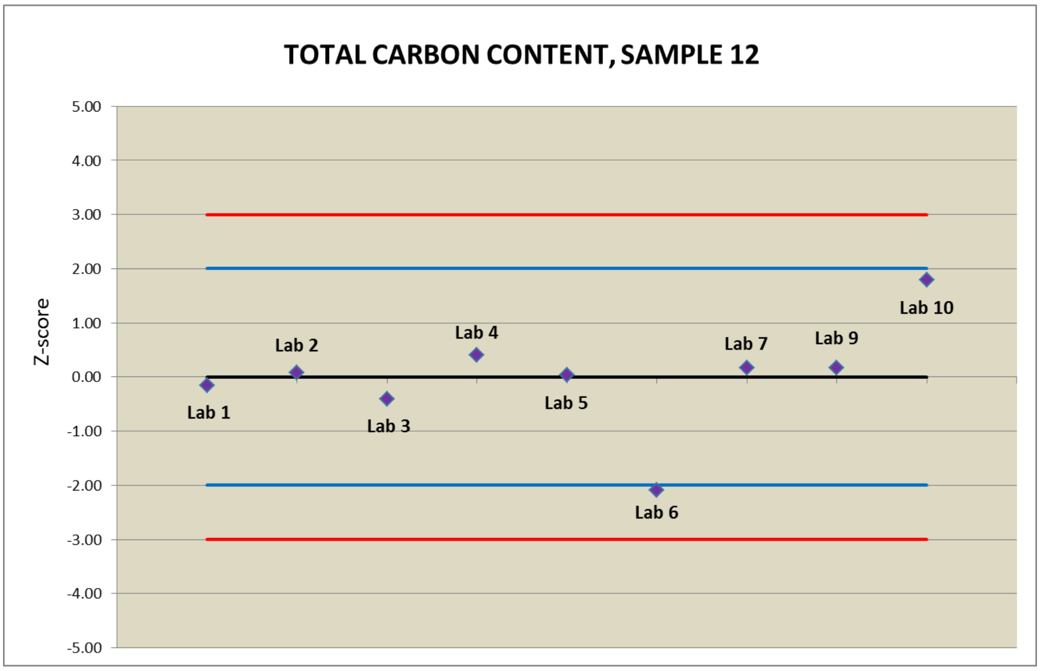
AVERAGE	39.5
STD	2.7
Min	34.4
Max	43.7



SAMPLE 12: Wooden particle board



AVERAGE	45.6
STD	2.1
Min	41.3
Max	49.4

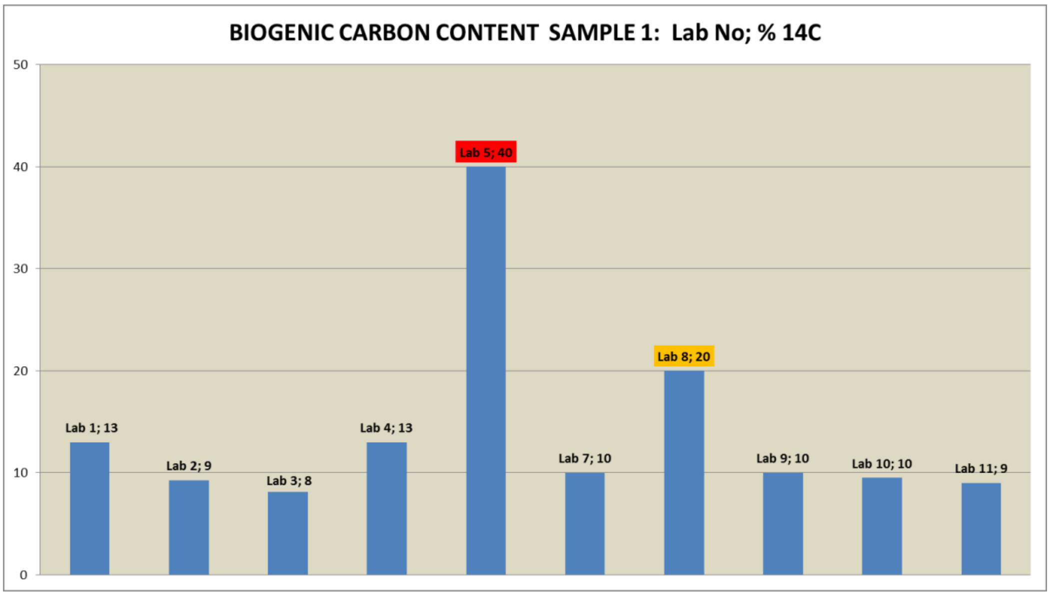


Appendix B: Biogenic carbon content and Z-scores for Samples 1-12

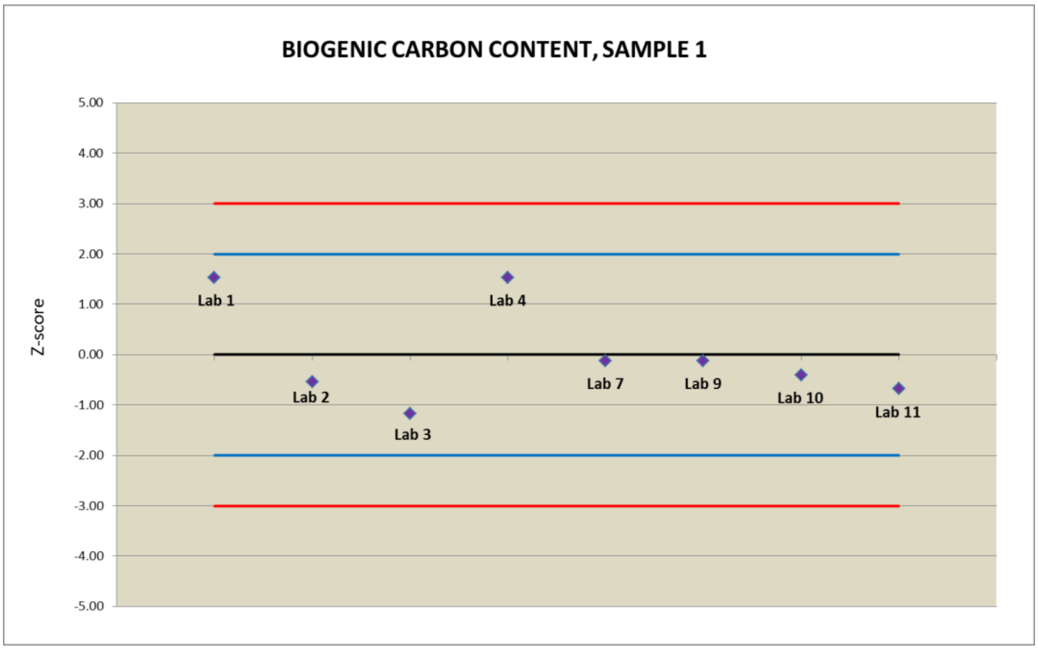
In this appendix the results of the measurements of the biogenic carbon content (as fraction of the total carbon content) are presented separately for each of the 12 samples. For each sample, the bar-plots give a comparison of the biogenic carbon content reported by all participating laboratories. Outliers and stragglers are included in these plots and are marked orange for the stragglers and red for the outliers. The data from product suppliers (when available) are included as well.

Next, Z-score plots are presented separately for each sample (for the calculations of Z-scores see paragraph 4). Outliers and stragglers were excluded when calculating the average numbers and the Z-scores. In this representation, for each individual sample, the Z-score plots indicate how far each laboratory is from the calculated average number that is depicted by the black line in the Z-score plots. Blue and red lines in the Z-score plots correspondingly indicate $2 \cdot S_R$ and $3 \cdot S_R$ borders, where S_R is the reproducibility standard deviation.

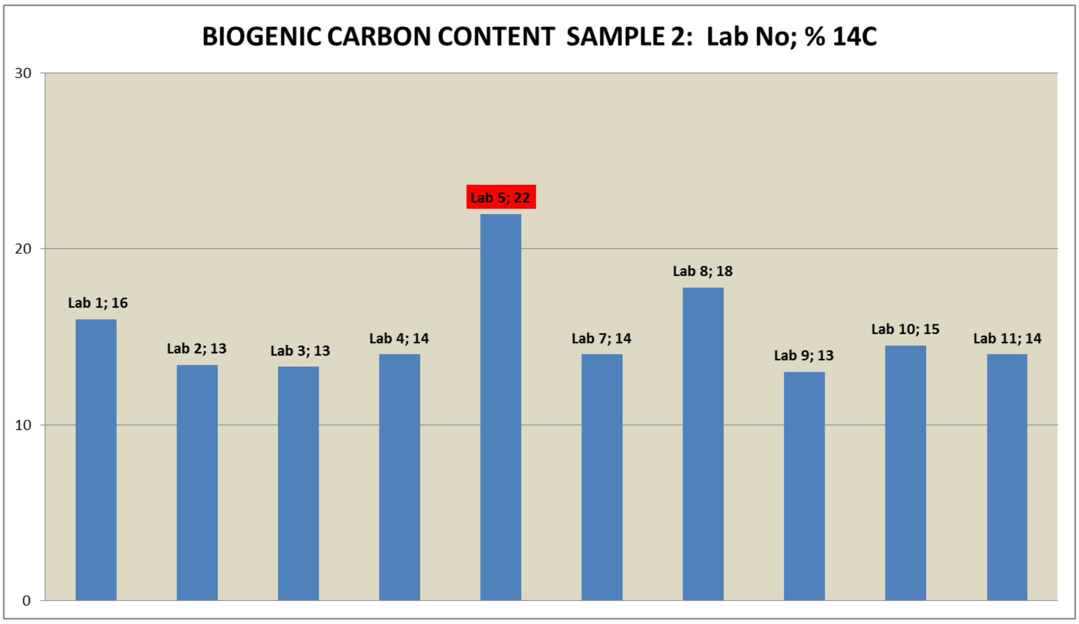
SAMPLE 1: White water soluble matt paint



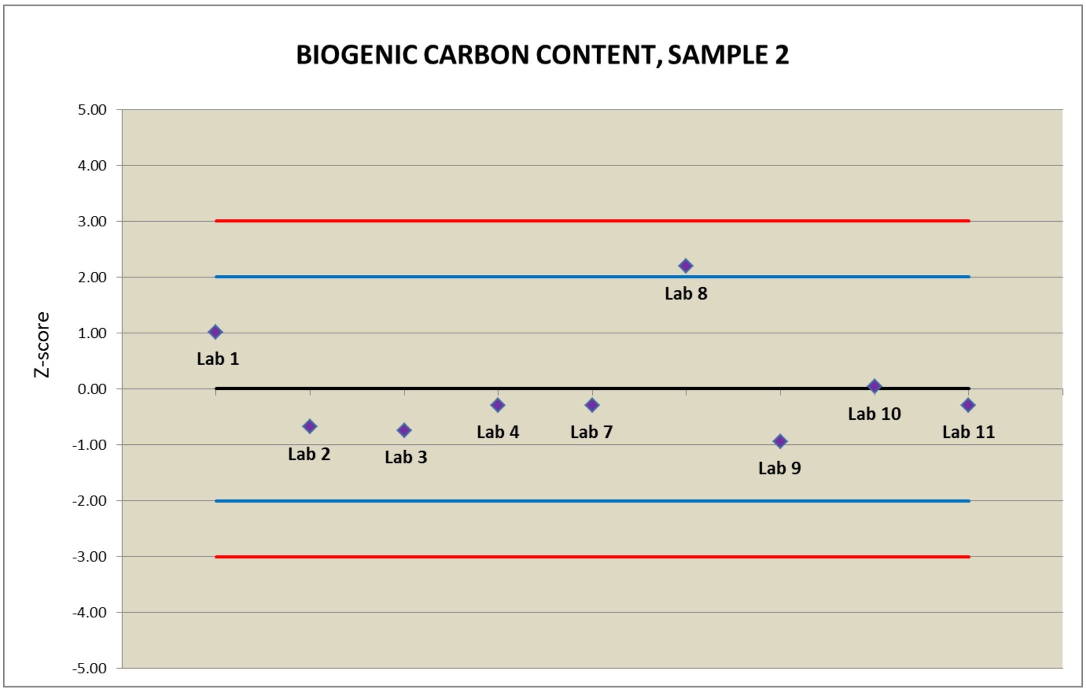
AVERAGE	10.23
STD	1.81
Min	8.10
Max	13.00



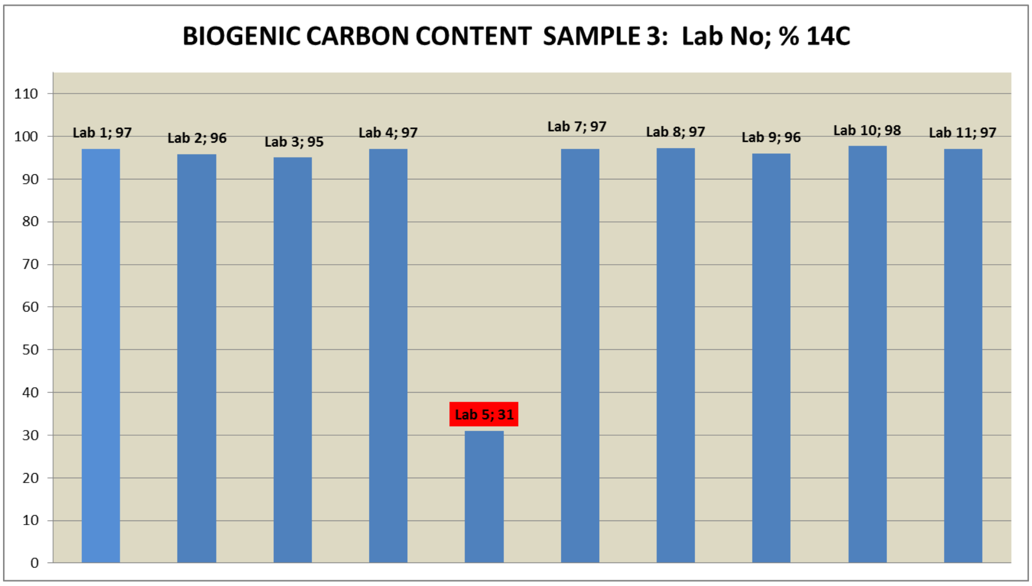
SAMPLE 2: White emulsion



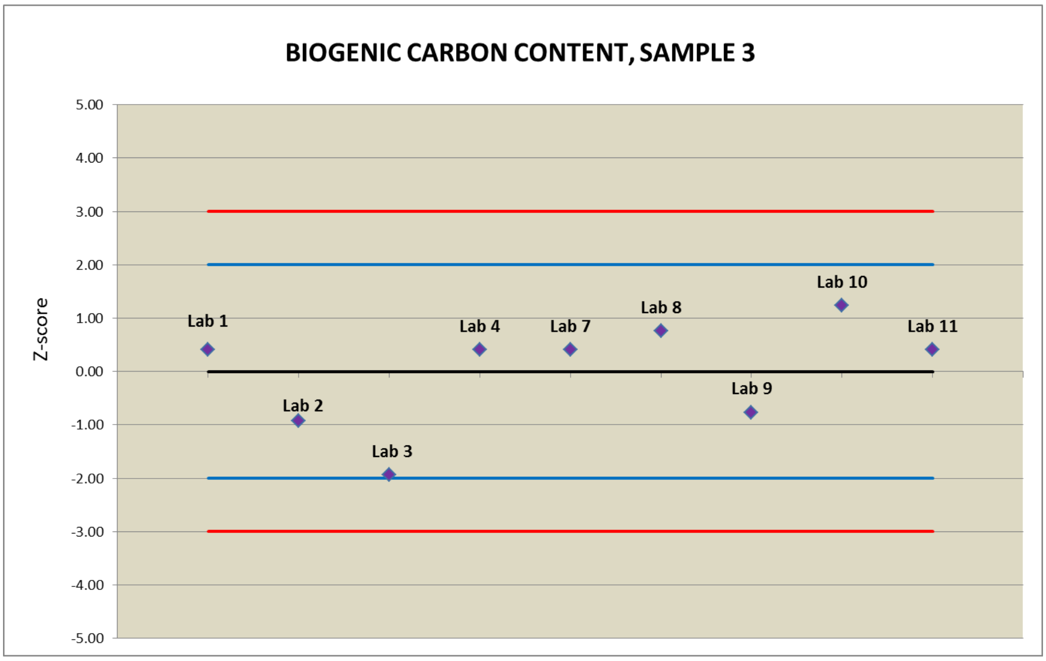
AVERAGE	14.44
STD	1.53
Min	13.00
Max	17.80



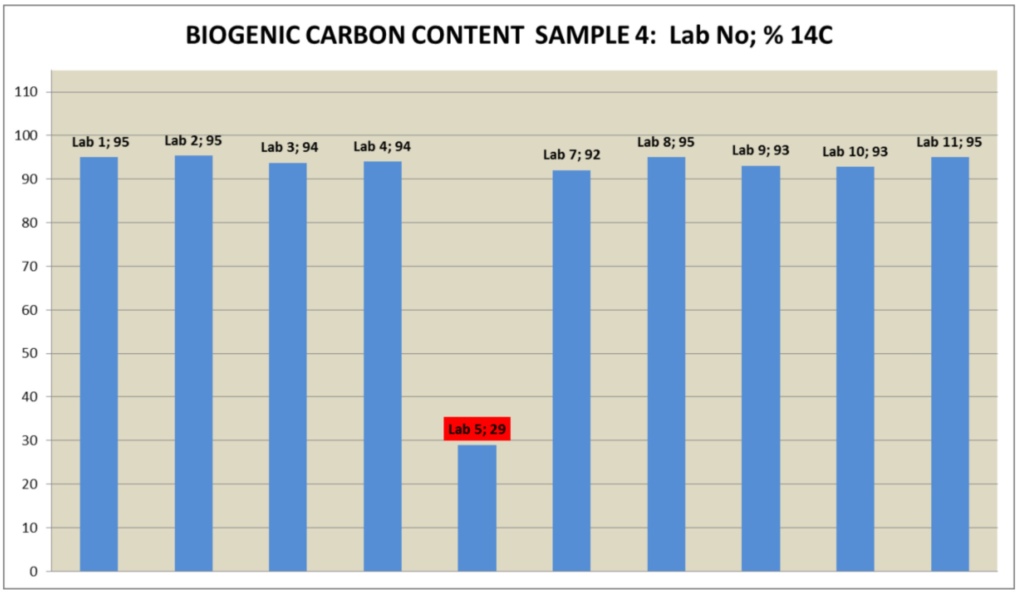
SAMPLE 3: White emulsion



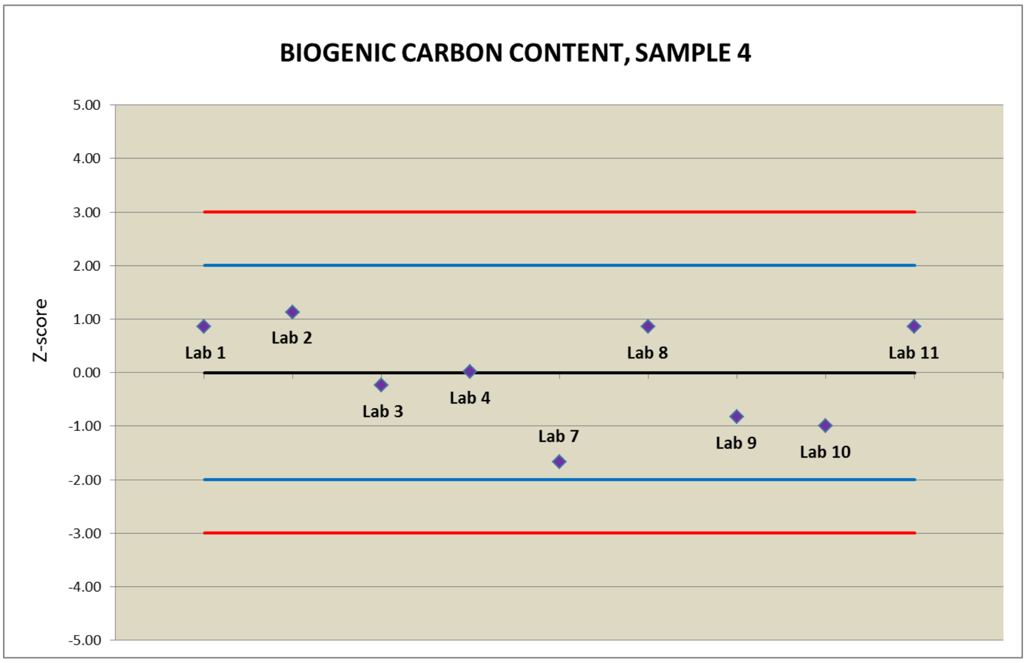
AVERAGE	96.65
STD	0.85
Min	95.00
Max	97.70



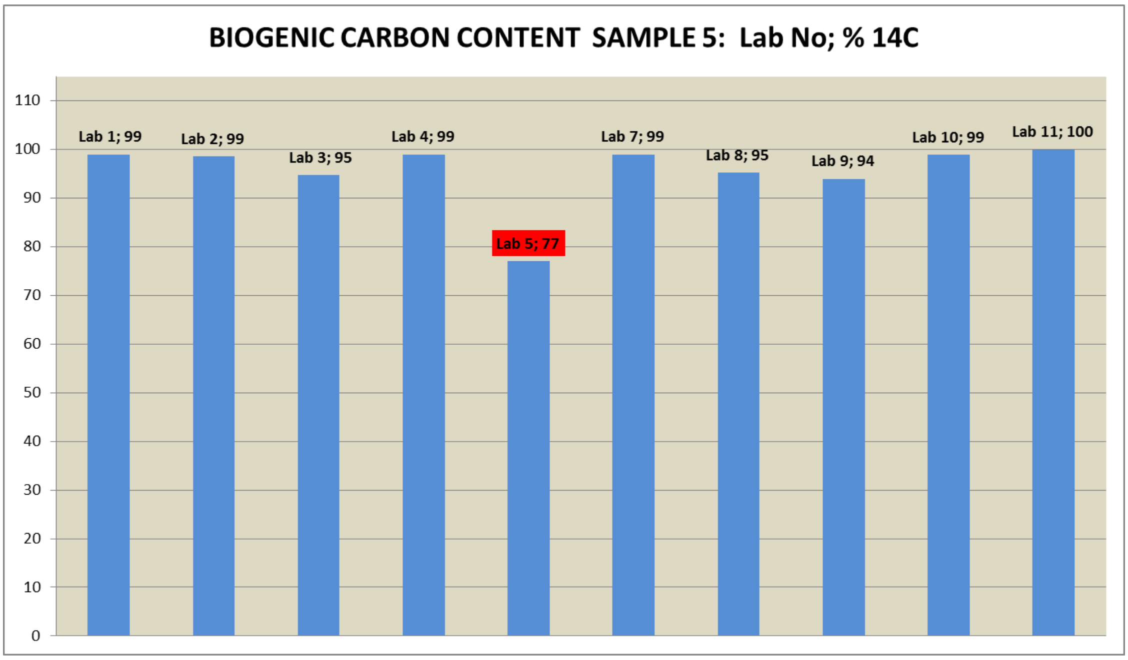
SAMPLE 4: Wheat straw panel



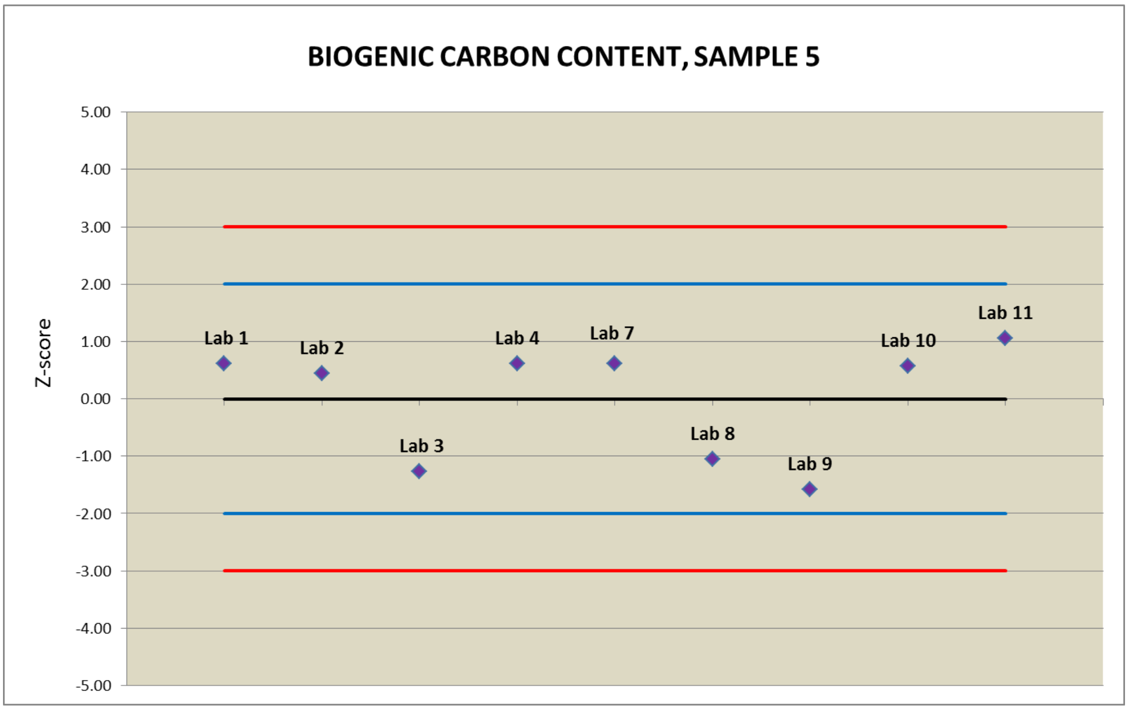
AVERAGE	93.98
STD	1.19
Min	92.00
Max	95.32



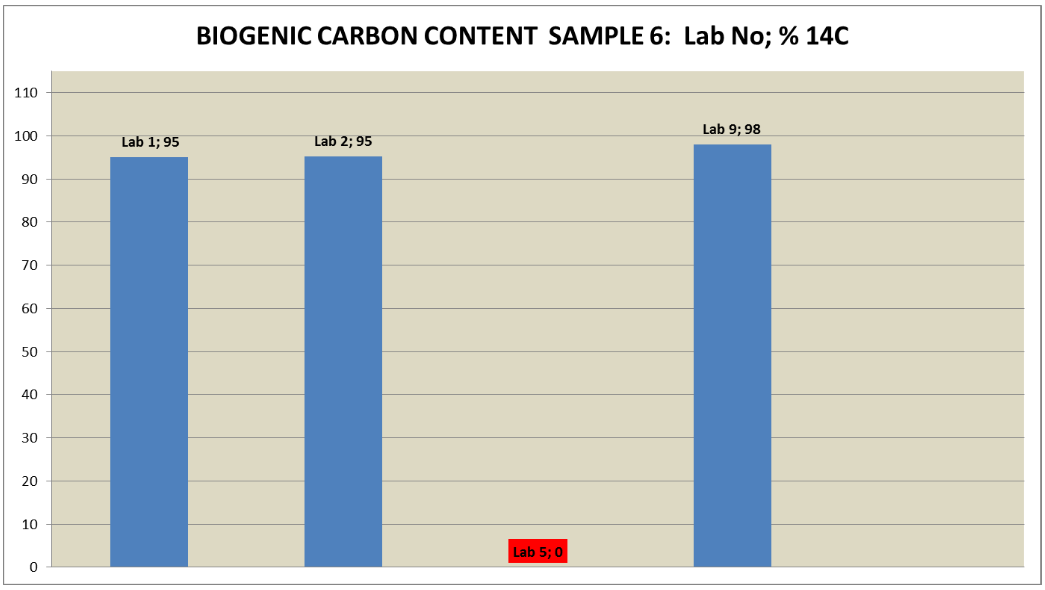
SAMPLE 5: Biodiesle



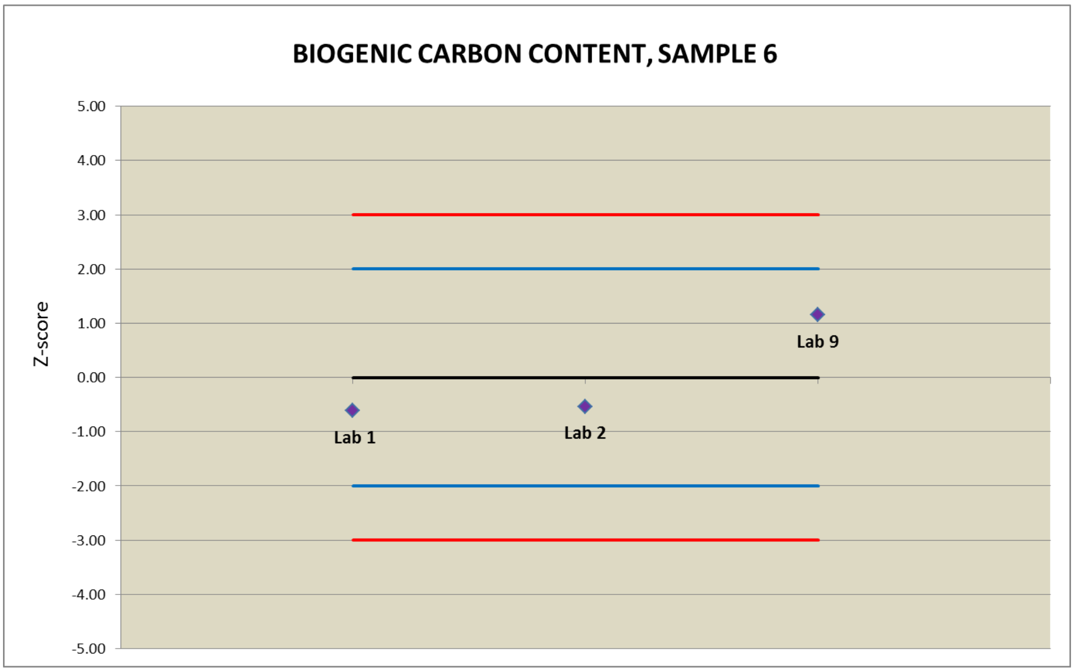
AVERAGE	97.60
STD	2.28
Min	94.00
Max	100.00



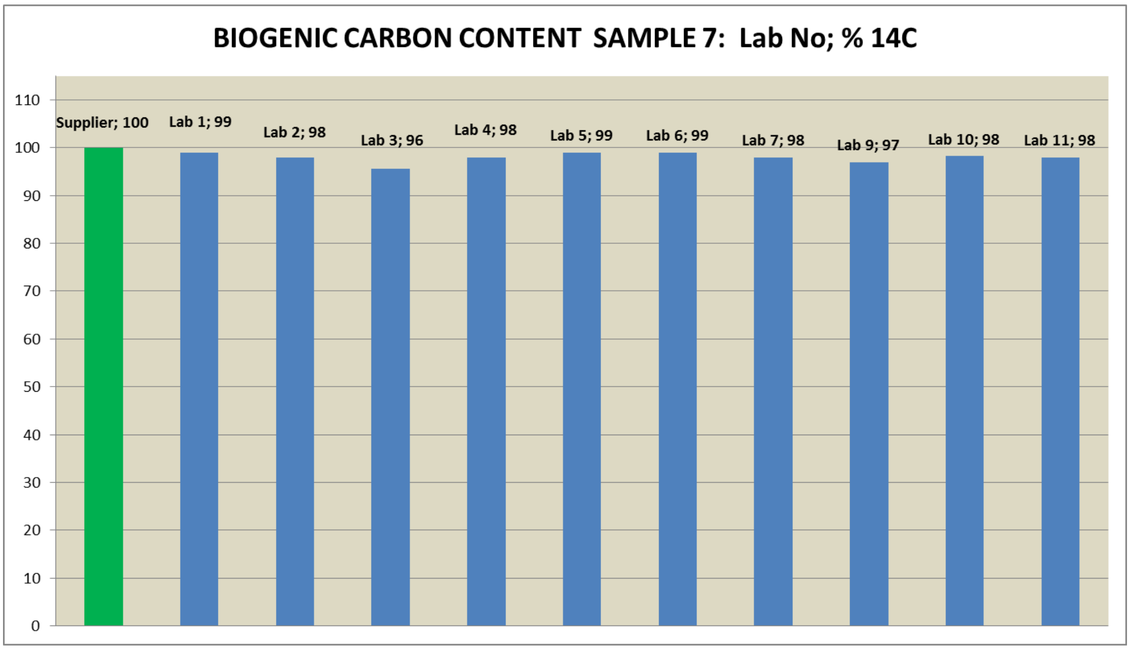
SAMPLE 6: Bio-gas



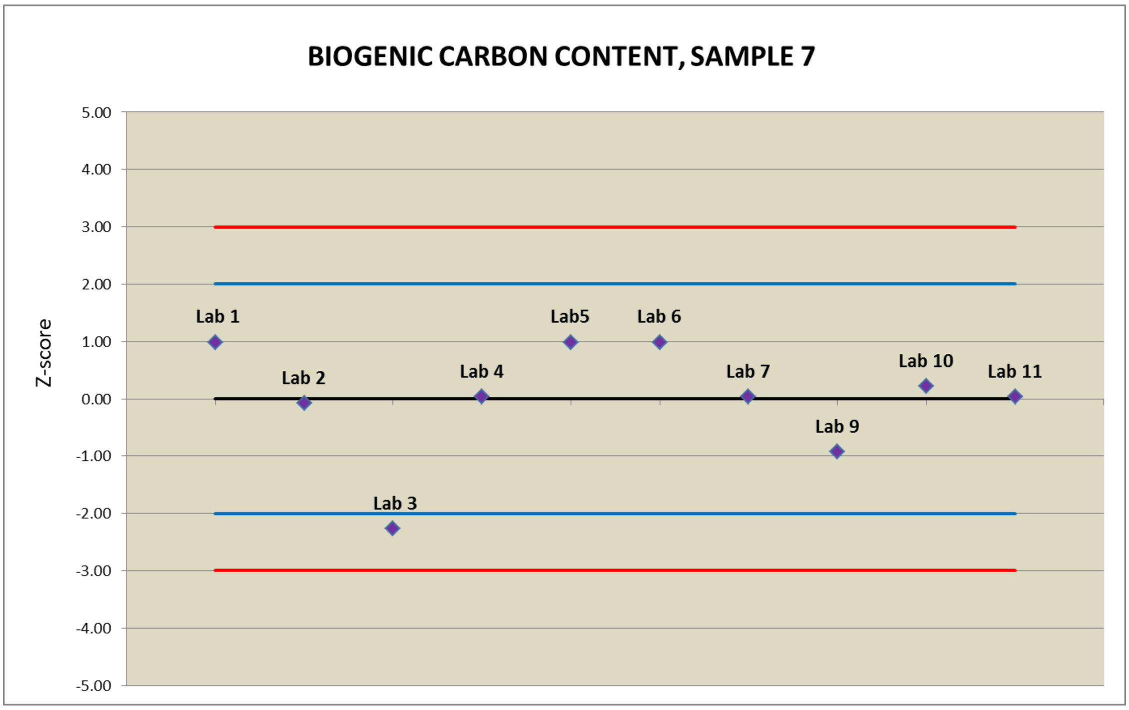
AVERAGE	96.05
STD	1.69
Min	95.00
Max	98.00



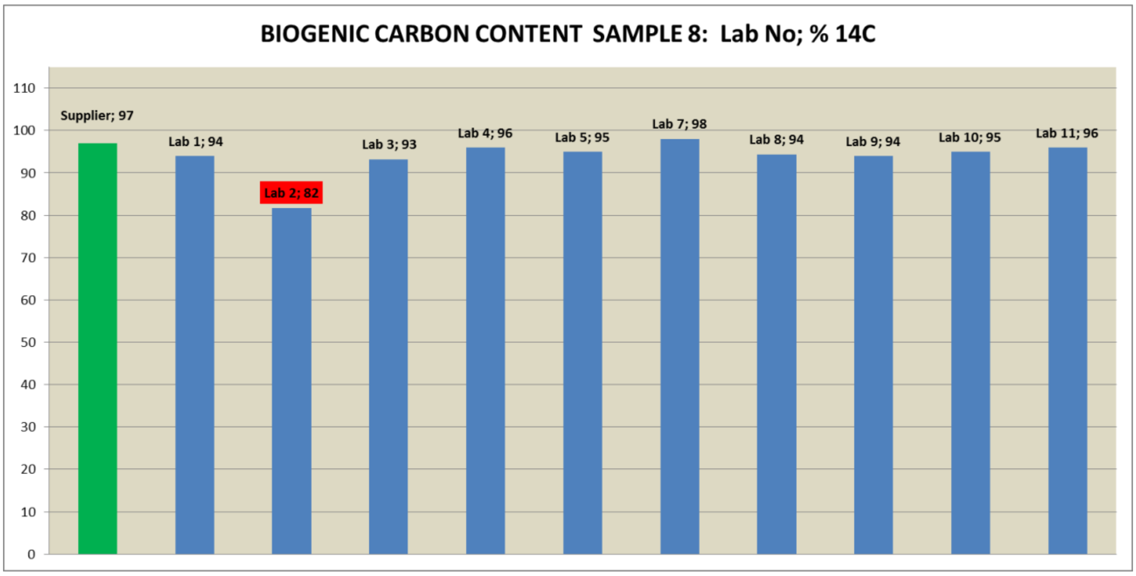
SAMPLE 7: White surfactant granules



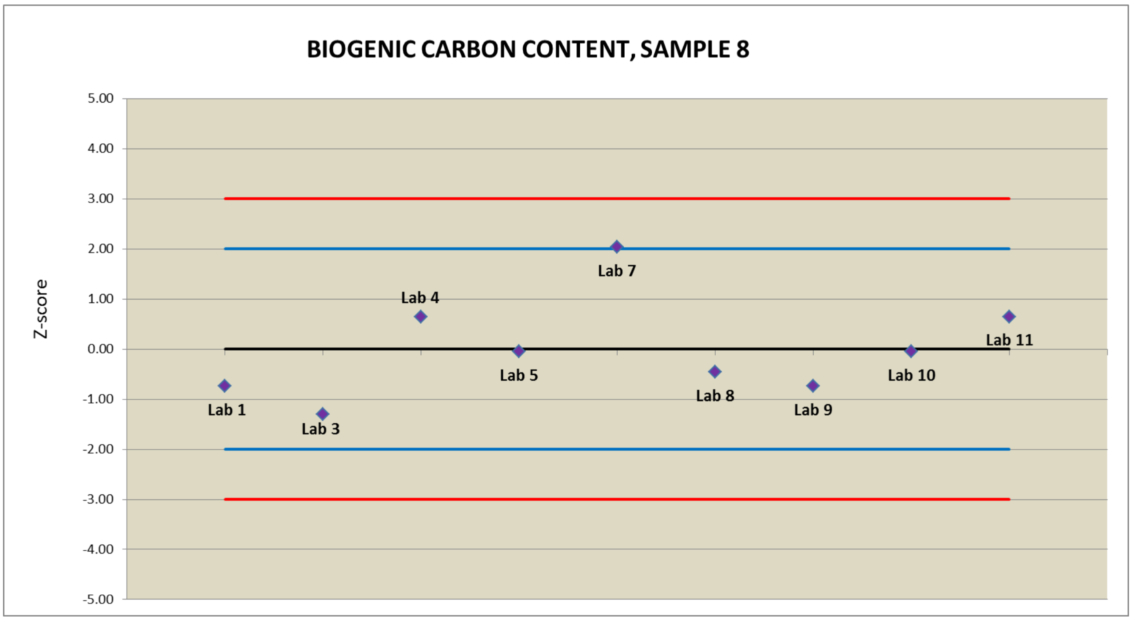
AVERAGE	97.97
STD	1.04
Min	95.60
Max	99.00



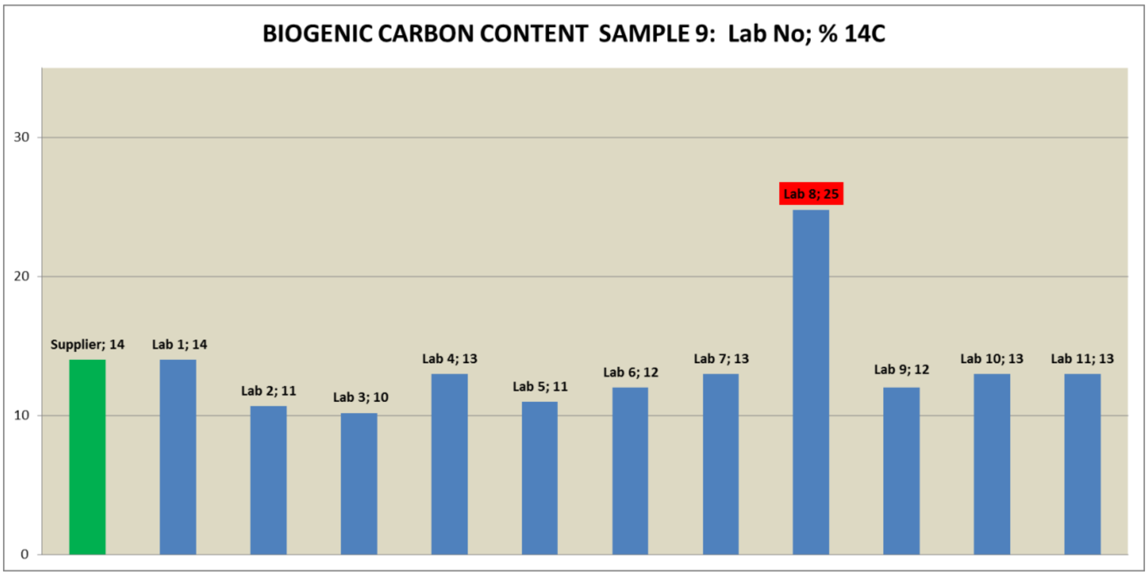
SAMPLE 8: Cosmetic emulsion



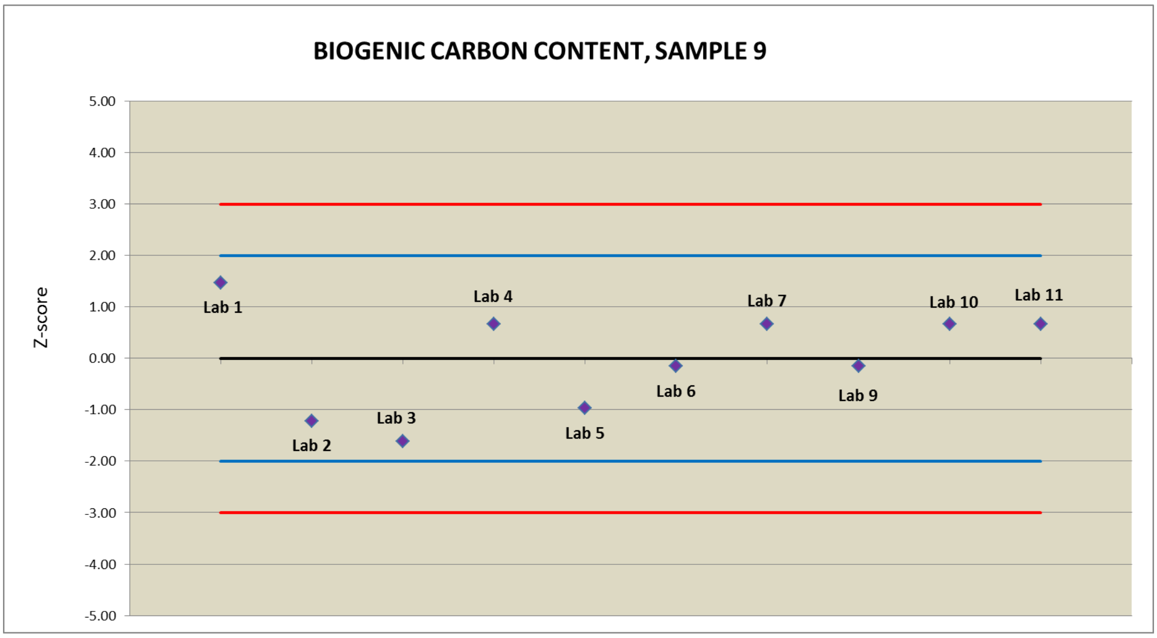
AVERAGE	95.07
STD	1.44
Min	93.20
Max	98.00



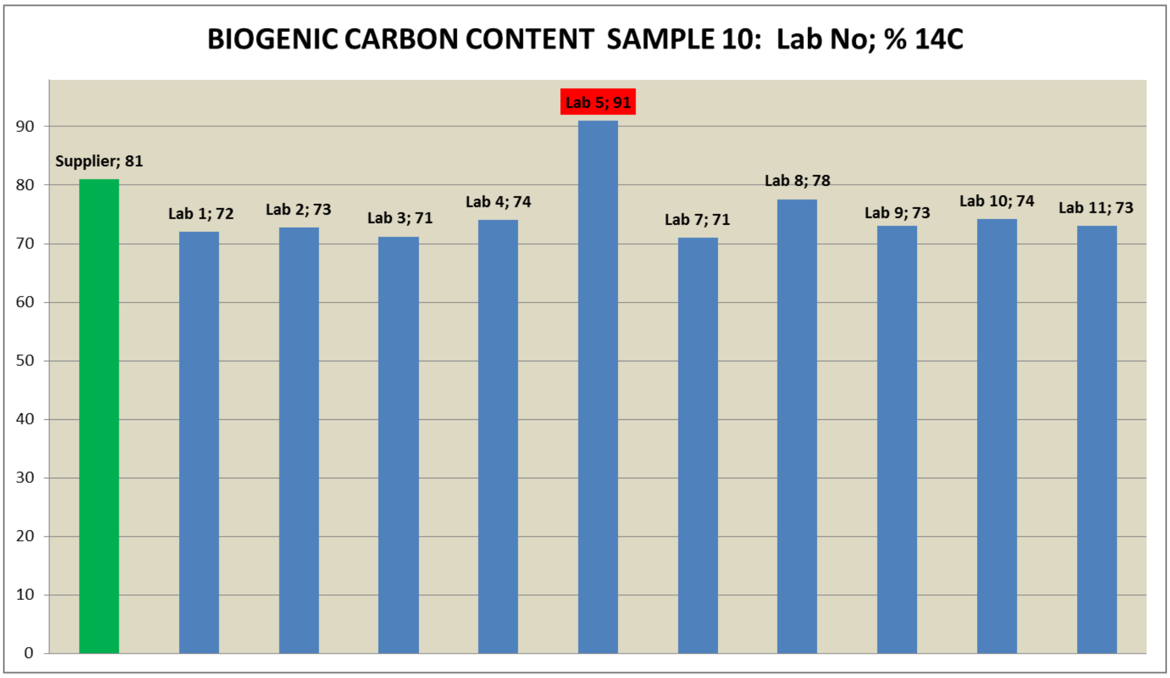
SAMPLE 9: Multilayer packaging film



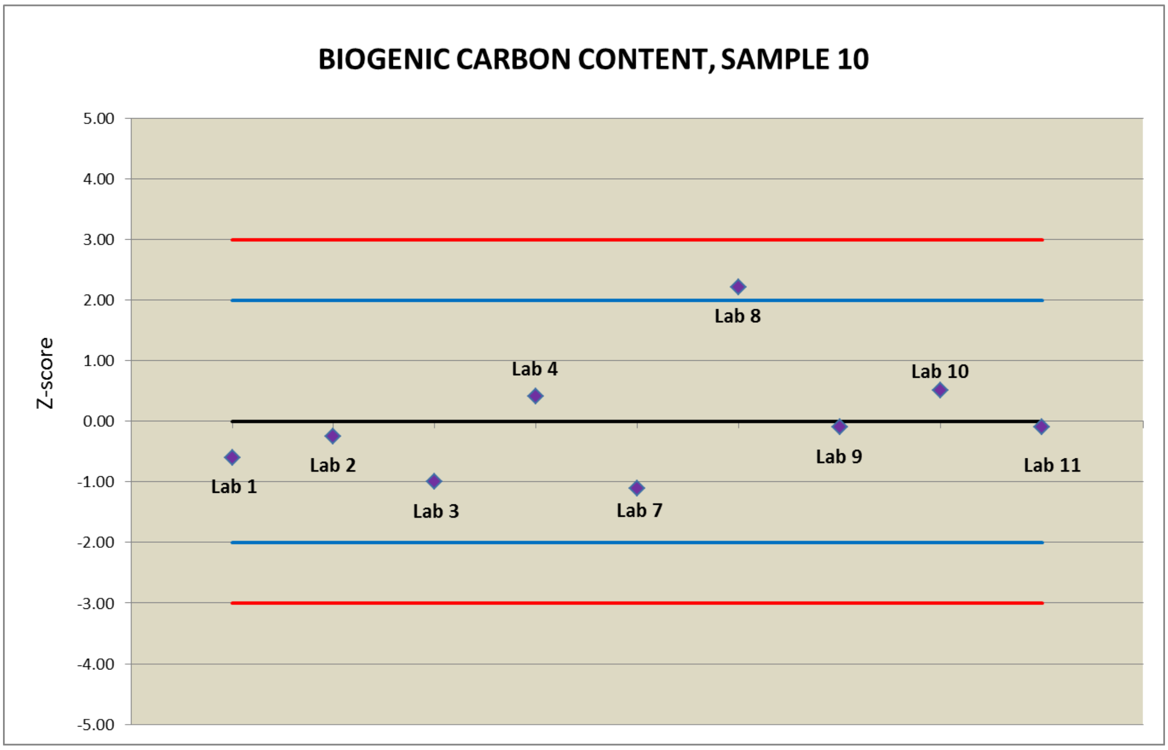
AVERAGE	12.19
STD	1.23
Min	10
Max	14



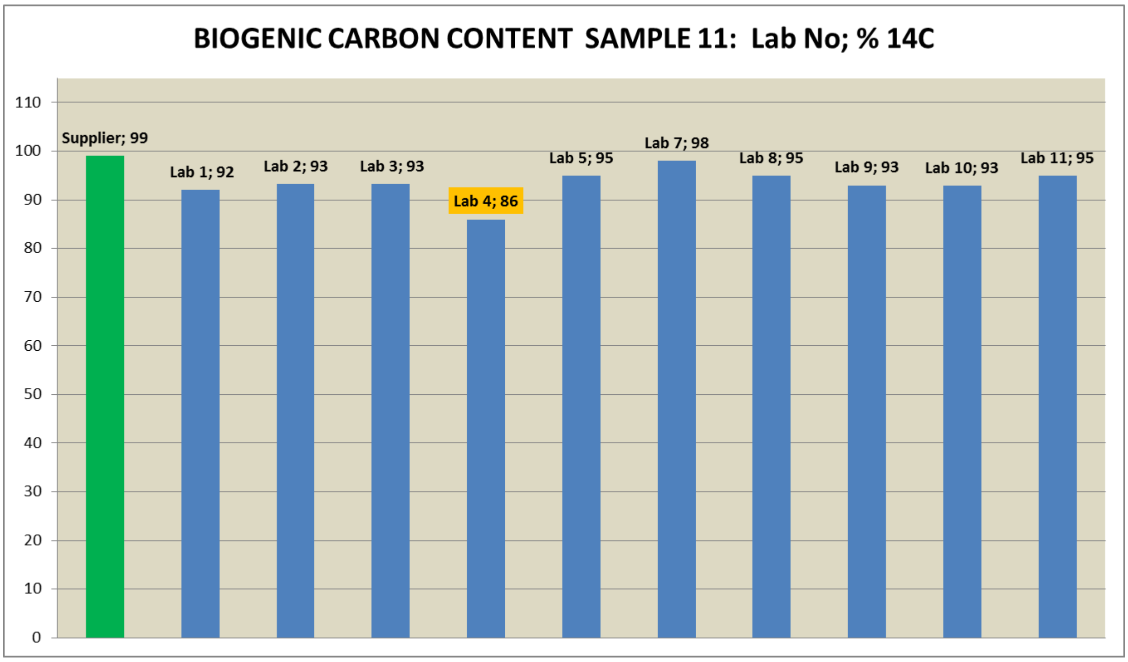
SAMPLE 10: Silk paint



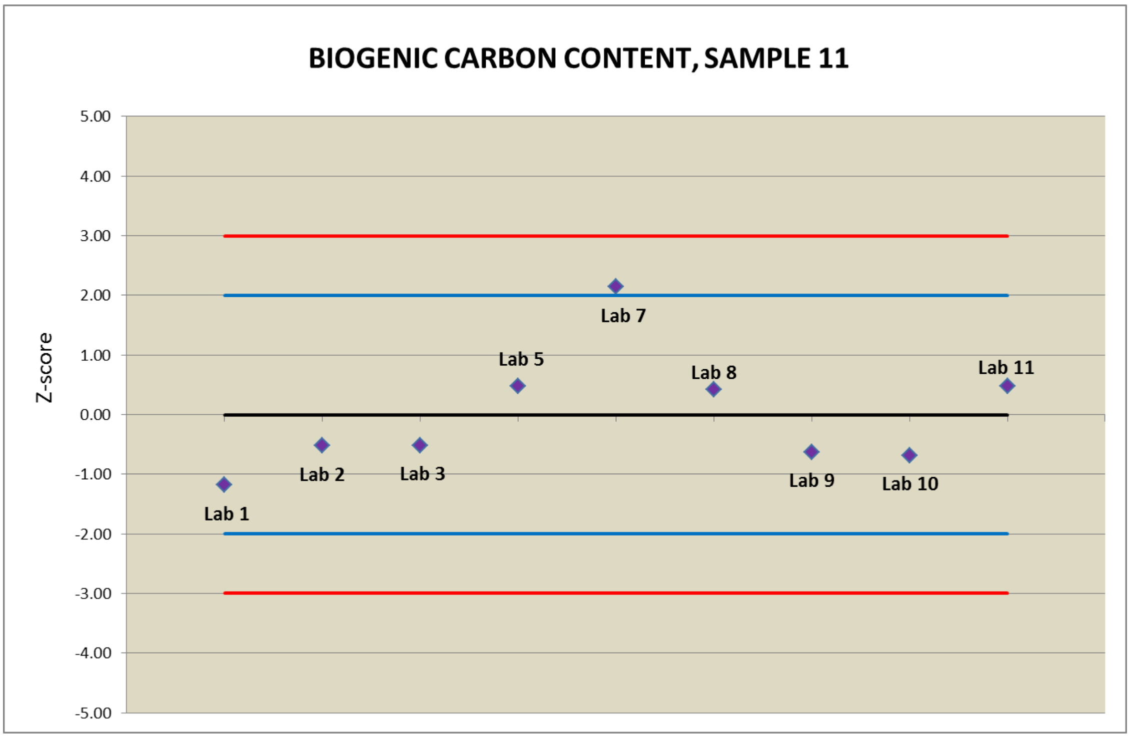
AVERAGE	73.19
STD	1.99
Min	71
Max	78



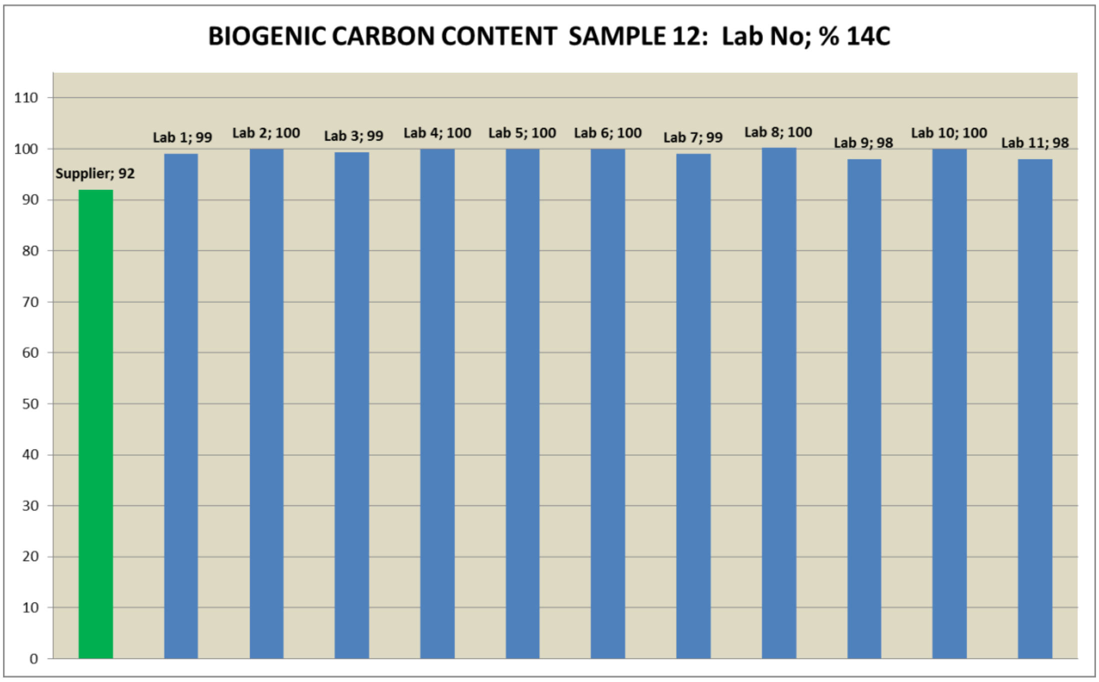
SAMPLE 11: Bio-based binder for paint



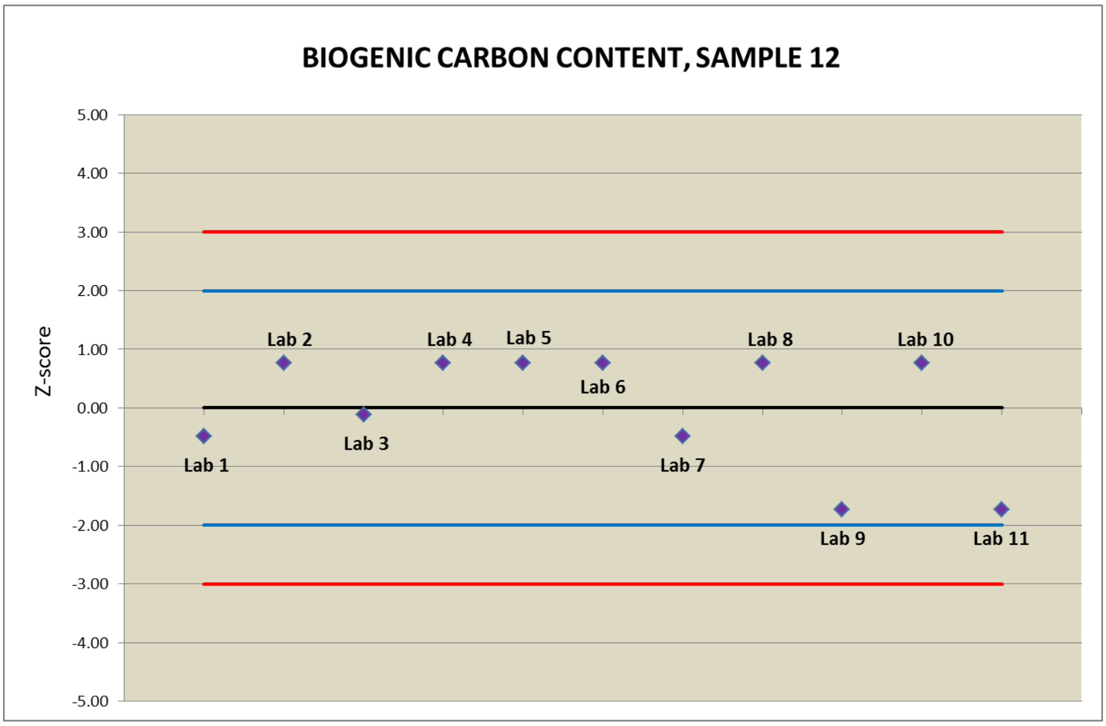
AVERAGE	94.13
STD	1.81
Min	92
Max	98



SAMPLE 12: Wooden particle board



AVERAGE	99.39
STD	0.80
Min	98
Max	100



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