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LABORATORY NAME:

LABORATORY NUMBER:

Amino Acid Racemization Proficiency Study

Report III: OSTRICH EGG SHELL (A)

June 2012

Acknowledgements.

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Contents

Tables

Figures

1 INTRODUCTION

1.1 Amino Acid Racemisation

Amino Acid racemization (or epimerization[i] for molecules with two carbon centres) is a diagenetic process that occurs naturally following protein synthesis. The process involves the slow inter-conversion between the two chiral forms of amino acids; the building blocks of proteins, from the Laevo (L-form) in life to the Dextro (D-form). Conversion of the L to D form continues until equilibrium is reached, for most amino acids this is usually equal to 1. This process can take many thousands of years, thus the D/L ratio value can be used as an indicator of time. This technique has been particularly successful in dating quaternary sediments using protein decomposition in fossil biominerals such as shell. The unique mineral crystalline structure of shells trap original proteins, with minimal loss and free from contamination.

The rates of racemization for the 20 or so different amino acids vary, are highly temperature dependent, matrix and species specific. Because the thermal history of a site is rarely known, it becomes difficult to determine precise age estimates. For this reason, most research tends to apply the technique as a relative stratigraphic tool within a defined locality using independently calibrated material; the assumption being that if all sites share the same temperature history, any observed D/L differences can be interpreted as relative age differences. Similarly, it becomes possible to use D/L values as indicators of relative temperature differences between same age sites, if independently dated using other appropriate techniques.

The last 30 years has seen significant changes in the analysis of amino acid racemization. Early research based on ion-exchange liquid chromatography (IE-LC) focused on the ratio between the D and L form of isoleucine but as methods developed, it became possible to detect and measure increasing numbers of amino acids, from six or seven using gas chromatography (GC) to ten or more routinely determined today using reverse-phase HPLC (rp-HPLC). These advances have continued to improve the precision in routine analysis and its acceptability as a valid dating method within the geochronology community. AAR now requires mg sample sizes, is relatively fast and with inexpensive preparation and analytical costs, is a useful screening method with the potential to provide age estimates that go far beyond current radiocarbon timescales, covering the entire quaternary period.

Nonetheless, AAR data is still often viewed dismissively. Important unaccounted differences between AAR age estimates and other dating methods have been previously reported (Wehmiller, 1992) with wide precision estimates for numerical ages up to 40-50% where the age equation was not calibrated locally, improving to 15% when it is (McCoy, 1987). More recently a value of 30% representing 53-142 years in Holocene shells has been reported following the removal of outliers (Kosnik et al., 2008).

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[i] Note; The more general term 'racemization' will be used throughout this report to refer to both racemization and epimerization.

Clearly, the accuracy of numerical age estimates relies heavily on the accuracy of analytical data. Wehmiller and Miller (2000) in their review of aminostratigraphic dating methods, report intra-laboratory precision estimates for repeated instrumental determinations of the same hydrolysate of 2%, for multiple analyses of different fragments of the same material, between 3-5%, whilst for multiple samples from the same sample location, between 5-10%. Previous inter-laboratory studies have focused on comparing individual laboratory precision estimates derived from replicate instrumental measurements (Wehmiller, 1984). These studies have demonstrated the variability in precision between different amino acids and methods. Whilst most laboratories report CV% values between 2-5%, there are often significant differences between laboratories that would result in substantial numerical age differences of 25% or greater, and call for the need for a common working standard with D/L reference values.

In spite of these efforts, there remains inconsistency in the use and expression of precision estimates, ambiguity in the reporting of uncertainty, and an absence of any assessment of method or laboratory bias, not least due to the absence of a suitable reference material. It is with regard to these issues that the current study has been undertaken and attempts to address.

Many laboratories continue to report uncertainty estimates as the CV of replicate instrumental measurements. Although analytical precision (i.e.; instrumental repeatability) is an important component of the overall uncertainty budget, it is usually amongst one of the smallest contributions and is often negligible compared to method and laboratory precision estimates. However, determination of method/laboratory precision through method validation or inter-laboratory collaborative trail, are outside the scope of this report.

Experience within other industry sectors has demonstrated, through regular participation in proficiency tests, that analytical performance improves over time. It is now nearly thirty years since the last inter-laboratory study was carried out using powdered fossil material (Wehmiller, 1984), and it is timely to coordinate a new inter-laboratory study in support of current methodologies.

1.2 Proficiency Testing

It has long been widely appreciated that participation in inter-laboratory studies is a valuable tool enabling method comparisons and development. Proficiency testing (PT) is a specific type of inter-laboratory evaluation providing an objective and formalized evaluation of accuracy against a consensus value enabling an objective comparison with other laboratories' data and is an important indicator of bias. Accuracy and by inference, performance, is characterized by elements of both precision and trueness. A laboratory may be inaccurate due to systematic bias effects, random error influencing poor repeatability, or both. In the absence of Certified Reference Materials (CRMs) for bias determination, participation in a proficiency test can provide a valuable alternative for laboratories.

Proficiency testing is commonly encountered in sectors that rely heavily on regulation and compliance such as medicine and public health, forensic science, chemical and geochemical analytical services, manufacturing industries, calibration and engineering, food and feed industries. Today more than 1,300 PT schemes worldwide are listed on the EPTIS[ii] website. Participation in such a scheme is also a requirement of analytical laboratories seeking accreditation to ISO 17025 (2005).

The regular analysis of an independent quality control material forms a valuable part of external quality control (EQC) enabling comparability on a much wider scale with other laboratories, analysts

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[ii] European Proficiency Testing Information Service; http://www.eptis.bam.de/en/about/what_is_eptis/index.htm

and methods. As such, it is an essential element of any laboratory's Quality Assurance (QA) programme, together with the use of validated methods and internal quality control (IQC) procedures.

Whilst performance in individual rounds can identify unexpected error influences needing investigation, long term trends are probably of greater value and can be observed using control charts (Thompson et al., 2006). The spread of results from a laboratory over a period of time should be compatible with that laboratory's own evaluation of uncertainty. The standard deviation of the differences between the laboratory values and the assigned values providing a means of evaluating the standard uncertainty (Eurachem 2000), see Section 6.2.2.

Test materials left over after the end of a proficiency test can also act as suitable matrix specific reference materials in the absence of CRMs. Because the value of the analyte has been determined by a consensus, it has minimal bias associated with it and a known uncertainty.

1.2.1 Organisation

This report is organized in to a number of sections. The next section, Section 2, details how test materials were prepared and distributed, and Section 3 presents the homogeneity data and discusses some of the issues encountered with the assessment of homogeneity for this test material. A summary evaluation of submitted results is presented in Section 4. Values for peak area and peak height together with concentrations and D/L values are tabulated with individual laboratory standard deviations, percentage relative standard deviations (RSD%) otherwise referred to as the coefficient of variation (CV%), instrumental replicate standard uncertainty estimates (u) representing precision from repeated measurements, (i.e.; instrumental repeatability) and the percentage relative standard uncertainty (RSU%). Section 5 assesses the accuracy of the results compared to the assigned value and calculates the relative percentage bias as an indication of performance. The last section, Section 6 then turns to the subject of measurement uncertainty and discusses the requirement for bias estimation in addition to precision estimates for uncertainty determination. The section demonstrates how proficiency test data can be used to derive indicative standard uncertainty contributions and values for combined and expanded uncertainty estimates. Finally method details as provided by the participants have been collated and together with the glossary of terms and symbols used in this report, relevant statistical tables and references, make up the Appendices at the end of the report.

2 TEST MATERIALS
Ostrich Egg Shell (A)

2.1 Preparation

The calcitic ostrich egg shell test material was prepared from a blown modern ostrich egg supplied by Oslinc Ostrich Farm, Boston in Lincolnshire, UK, in 2010. A section of the egg shell was broken into pieces and approximately 50 g was cleaned with repeated washing in ultrapure water using a sonicator. Rehydrated shell membrane lining was removed by peeling and scraping and further washed until the water remained clear. The cleaned ostrich egg shell was then lightly covered and left to air dry for 48 hours. The broken shell pieces were placed on a flat heat-proof dish and heated in the oven for 8 hours at 140 ^{o}C. After cooling, pieces of the heated shell were lightly milled using short bursts of an electric coffee mill to avoid heating of the motor and blade. The reduced fragments and course powder were further ground using a sterile pestle and mortar and sieved, to ≤ 250 µm before finally being tumble-blended overnight on a roller mixer.

Half the heated, powdered ostrich egg shell was bleached with intermittent shaking, for 48 hours using 50µl of 12% NaOCl per mg of powder. The bleach was removed and the powder washed with ultrapure water up to six times using a vortex mixer followed by centrifugation to pellet the solids in between washes. A final wash with methanol to remove any remaining water was carried out before the material was again lightly covered and left to air dry.

Individual 20 mg sub-samples of the cleaned, bleached and dried ostrich egg shell powder were weighed into sterile glass vials and labelled as Ostrich Egg Shell (A) (OES (A)). The remaining half of the heated, powdered but unbleached material, was also weighed (20 mg sub-samples) into sterile glass vials and labelled as Ostrich Egg Shell (B) (OES (B)). Both sets of test material were stored at room temperature prior to distribution.

2.2 Homogeneity

Ten randomly selected test materials were sub-sampled to give 10 duplicate samples (10 x a and b), which were then analysed for total hydrolysable amino acids (THAA) using reverse phase HPLC (rpHPLC) according to the standard method (Kaufman and Manley W.F., 1998). The results, together with their statistical evaluation, are given in Section 3.

2.3 Distribution

Participants were previously asked to notify the organizer with details of their proposed analytical method and were sent the appropriate number of individual test materials necessary to give sufficient bulk material required by the different methods. Those using rpHPLC were sent a single individually numbered 20mg test material, those using ion-exchange HPLC (HPLC-IE) were sent three individual test materials (60mg total) and those using gas chromatography (GC) were sent ten individual test materials (200mg total). Participants receiving multiple test materials were asked to pool the contents to get the required quantity rather than simply having a larger sample sent because of the risk of heterogeneity in larger sub-samples. This way, a defined minimum measure of homogeneity could be assured between individual sub-samples of a specified weight, which would not be lost when pooled.

Test materials were dispatched to eight laboratories located around the world on 15 July 2010.

Due to the small number of participants in the study, additional sets of test materials were provided to those laboratories who had more than one instrument, those using more than one method and those who had more than one member of staff available to carry out the analysis. As a result this increased the possible number of sets of results up to twenty three.

2.4 Result Submission

Participants were asked to submit results and method information on electronic documents sent following dispatch and no later than October 2010. The final set of results was submitted mid-December but three participants were unable to return any results on this occasion due to instrumental difficulties or other commitments. A total of fifteen sets of results were submitted.

Whilst the original intention of this study was to determine performance for only D/L amino acid values, a number of laboratories also asked to submit raw chromatogram data. Consequently, a results proforma was prepared enabling the submission of peak area and height data, together with concentrations and D/L values. Participants were asked to indicate their primary means of determination, i.e.; using peak areas, heights or concentrations. Due to the delay in results being submitted and the time required in assessing the data, the additional information has been summarized and tabulated in Section 4 but not evaluated. Where more than one replicate value was submitted, **instrumental repeatability** standard uncertainty estimates have been determined and plotted to demonstrate the effect of the expanded uncertainty at a 95% confidence level (2 std deviations approximately) on the mean value. Where results were submitted as the mean and standard deviation, these values have been used for the calculation of the standard uncertainty directly.

One laboratory provided free amino acid data (FAA) but these have not been assessed or tabulated on this occasion. In this report only data given for the total hydrolysable amino acid fraction (THAA), have been evaluated. Instrumental replicate measurements provided by individual laboratories have been averaged as necessary to give a single value for each amino acid in the test material supplied. These are tabulated in Section 5, together with an evaluation of performance, assessed as the relative percentage bias, which are also presented as histograms at the end of the section.

Each set of results was given a unique laboratory number. The analytical methods used by each participant are summarised in Appendix I.

3 HOMOGENEITY
Ostrich Egg Shell (A) Test Material

3.1 General Procedure

The purpose of carrying out homogeneity testing, is to prove that any variation in composition between individual test materials, characterized by the sampling standard deviation (s_{sam}) is negligible compared to the variation in measurement determinations carried out by participants of the proficiency test. Due to the time and expense of preparing homogeneous test materials and carrying out the analysis, it is reasonable to start with the assumption that test materials are homogeneous and by carrying out homogeneity testing we are looking for evidence of heterogeneity, rather than vice versa. The following procedure for the assessment of homogeneity follows that given in the standard ISO 13528:2005, and the 2006 IUPAC International Harmonized Protocol (Thompson et al).

It is recommended that ten (and no fewer than seven) randomly selected prepared and packaged test materials are selected at random using a random number generator. Each sample is then individually homogenized and two separate portions are removed and labeled 1a and 1b; 2a & 2b;….10a & 10b etc. Each individual sub-sample is then prepared according to the appropriate method and analysed in a random order under repeatability conditions, (i.e.; at the same time or in as short a time as possible, as a single batch on the same day by the same analyst on the same instrument etc).

Resulting data should be scrutinized first for obviously anomalous values eg values greater or less than 10 times the average. It is helpful to plot data in run order to identify trends, stability issues or measurement problems. However, assuming no problems are identified the data should be sorted and sub-samples re-paired to undergo the following statistical evaluation.

3.1.1 Statistical analysis.

a) Data are initially subjected to a Cochran's outlier test.

The Cochran's test statistic is determined by the ratio of the maximum squared difference to the sum of squared differences;

$$C = {D_{max}^2}/{\sum D_i^2}$$

Where; C is the Cochran's statistic,

D_{max} is the largest difference between duplicates, and

D_i is the difference between each pair of duplicates.

The C-value is then compared against tabulated critical values based on the required confidence level and the degrees of freedom, *m-1*, where *m* is the number of duplicate pairs. If $C > C_{crit}$, the pair is identified as a Cochran's outlier and removed from the data set.

b) Evaluation of Analytical Variance

Occasionally, genuine inhomogeneity between samples is missed due to large within-sample analytical variances, i.e.; between the two sub-sample values (eg; 1a & 1b). This can mask significant between-sample differences (eg; 1 - 10). It is therefore recommended to evaluate the analytical precision first to ensure that the method is sufficiently precise to detect inhomogeneity.

Data are assessed using a one-way ANOVA to estimate the analytical variance.

The analytical variance $s_{an}^2 = MS_w$ where MS_w= within groups mean square.

Note how s_{an} is analogous to the repeatability standard deviation, s_r in Section 4.1

Satisfactory analytical precision is assumed if the analytical deviation is less than half the target value for standard deviation (σ_p) for the proficiency test (Fearn and Thompson, 2001);

i.e.; $s_{an}/\sigma_p < 0.5$

Note; due to the absence of an external target value for standard deviation (σ_p), a target value for homogeneity (σ_h) has been determined such that $s_{an}/0.5 = \sigma_h$

c) Evaluation of Sampling Variance.

The sampling variance $s_{sam}^2 = \frac{MS_b - MS_w}{2}$ where MS_b= between groups mean square.

Or as $s_{sam} = 0$, if the above estimate is negative (Fearn & Thompson, 2001)

Note how s_{sam} is analogous to the between-sample standard deviation, $s_L$in Section 4.1.

Calculate the permissible sampling variance $s_{all}^2 = (0.3 \times \sigma_p)^2$

Calculate the critical value (c) for the test using tabulated values for F_1 and F_2 (ISO 13528:2005, Thompson et al; 2006, Fearn and Thompson; 2001).

$$c = F_1 s_{all}^2 + F_2 s_{an}^2$$

If $s_{sam}^2 < c$, the sampling variance has not exceeded the allowable fraction of the target standard deviation. There is no evidence of inhomogeneity and the test has been passed.

3.2 Evaluation of Ostrich Egg Shell (A) Test Material Homogeneity Data

Ten test materials were selected at random from the bulk of previously prepared individual test materials. Each test material was divided into two sub-samples and prepared according to the standard procedure prior to hydrolysis for total hydrolysed amino acids. The twenty individual sub-samples where then randomized and analysed as a single batch under repeatability conditions using reverse-phase HPLC.

Sub-samples 6b and 8b dried out and were lost during hydrolysis. The D/L results for the eighteen remaining sub-samples for each amino acid were plotted in run order to identify trends or problems with the data and are shown in Figure 3.1.

For all amino acids, results for sub-samples 6a and 8a were removed as they were paired with sub-samples lost during hydrolysis. For glutamic acid / glutamate and serine, sub-samples 7a and 7b were identified as Cochran's oultiers and also removed from the statistical evaluation.

The D/L results and statistical evaluation are given in Table 3.1. Removed values and those identified as outliers have been coloured red in the tables. Figure 3.2 shows the paired D/L values for each amino acid. Outliers that were removed from the statistical evaluation are shown as empty symbols on the charts.

In all cases, σ_h, the target standard deviation (for sufficient homogeneity), was set as the minimum value necessary to ensure fitness-for-purpose, i.e.; that σ_h was at least twice the analytical precision (repeatability) and that the allowable sampling variance was sufficient to accommodate the observed between-sample differences.

Table 3.1: Homogeneity D/L Values for Ostrich Egg Shell (A) Test Material

sample id	Asx D/L		Glx D/L			Ser D/L			Arg D/L		Ala D/L	
	replicate 1	replicate 2	replicate 1	replicate 2		replicate 1	replicate 2		replicate 1	replicate 2	replicate 1	replicate 2
1	0.373	0.374	0.093	0.093		0.325	0.324		0.146	0.127	0.112	0.107
2	0.377	0.373	0.094	0.093		0.325	0.322		0.124	0.146	0.105	0.109
3	0.381	0.372	0.094	0.093		0.328	0.327		0.147	0.145	0.109	0.108
4	0.377	0.375	0.094	0.094		0.327	0.329		0.145	0.127	0.110	0.106
5	0.379	0.375	0.094	0.094		0.324	0.325		0.148	0.146	0.108	0.107
6	0.380		0.095			0.330			0.144		0.108	
7	0.381	0.368	0.095	0.093	C	0.328	0.319	C	0.148	0.147	0.110	0.111
8	0.384		0.094			0.327			0.129		0.107	
9	0.370	0.371	0.094	0.094		0.322	0.323		0.147	0.144	0.109	0.108
10	0.379	0.381	0.095	0.095		0.326	0.327		0.129	0.127	0.106	0.105
mean, N	0.375	16	0.094	14		0.325	14		0.140	16	0.108	16
origin of target sd (σ_h)	perception		perception			perception			perception		perception	
abs. target sd (σ_h) & as RSD%	0.0086	2.3	0.0011	1.2		0.0029	0.9		0.0173	12.3	0.0041	3.8
s_{an}	0.0043		0.0003			0.0011			0.0086		0.0020	
s_{an} / σ_h	0.4931		0.2650			0.3855			0.4991		0.4885	
s_{an} / σ_h <0.5?	yes		yes			yes			yes		yes	
s_{sam}^2	0.00E+00		3.55E-07			3.17E-06			1.43E-05		3.42E-07	
σ_{all}^2	6.71E-06		1.14E-07			7.71E-07			2.68E-05		1.52E-06	
critical	3.61E-05		3.67E-07			3.44E-06			1.47E-04		8.10E-06	
s_{sam}^2<critical?	**ACCEPT**		**ACCEPT**			**ACCEPT**			**ACCEPT**		**ACCEPT**	

Table 3.1: Homogeneity D/L Values for Ostrich Egg Shell (A) Test Material (continued).

sample id	analyte							
	Val D/L		PheD/L		D-Aile/L-Ile		Leu D/L	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	0.034	0.031	0.084	0.082	0.036	0.038	0.068	0.067
2	0.031	0.034	0.082	0.081	0.035	0.034	0.068	0.068
3	0.033	0.034	0.083	0.085	0.038	0.037	0.069	0.069
4	0.031	0.031	0.083	0.083	0.037	0.035	0.069	0.068
5	0.032	0.033	0.083	0.083	0.037	0.037	0.069	0.068
6	0.032		0.083		0.036		0.069	
7	0.031	0.032	0.084	0.082	0.036	0.037	0.069	0.069
8	0.031		0.083		0.038		0.071	
9	0.033	0.031	0.082	0.082	0.035	0.035	0.068	0.067
10	0.031	0.031	0.083	0.083	0.036	0.036	0.069	0.068
mean, N	0.032	16	0.083	16	0.036	16	0.068	16
origin of target sd (σ_h)	perception		perception		perception		perception	
abs. target sd (σ_h) & as RSD%	0.0024	7.5	0.0017	2.1	0.0016	4.3	0.0011	1.6
s_{an}	0.0012		0.0008		0.0008		0.0005	
s_{an} / σ_h	0.4966		0.4860		0.4963		0.4759	
s_{an} / σ_h <0.5?	yes		yes		yes		yes	
s_{sam}^2	6.51E-08		1.67E-07		6.92E-07		3.81E-08	
σ_{all}^2	5.18E-07		2.72E-07		2.17E-07		1.08E-07	
critical	2.81E-06		1.44E-06		1.18E-06		5.56E-07	
s_{sam}^2<critical?	ACCEPT		ACCEPT		ACCEPT		ACCEPT	

Figure 3.1: Homogeneity Amino Acid D/L Values in Analytical Sequence Order.

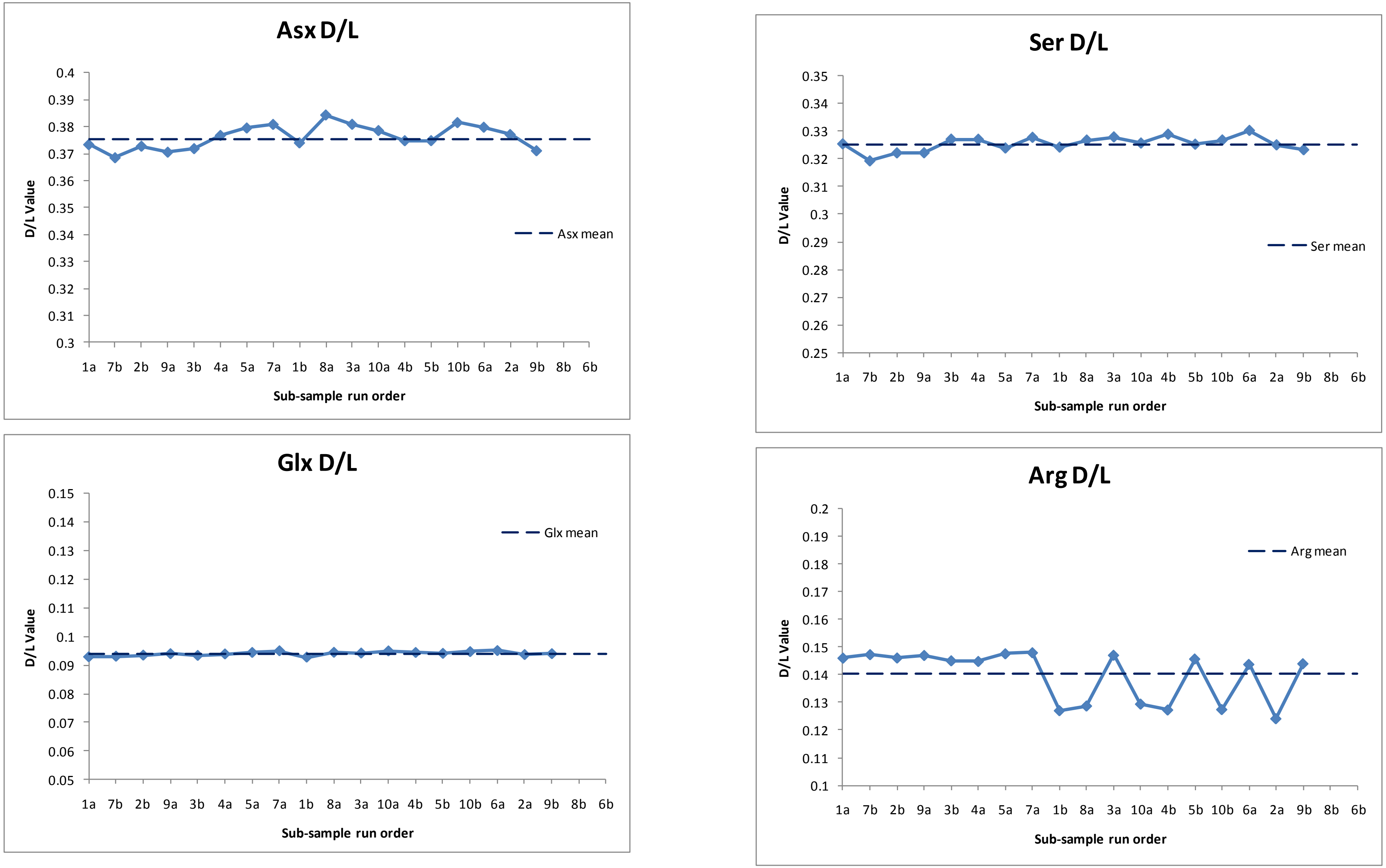
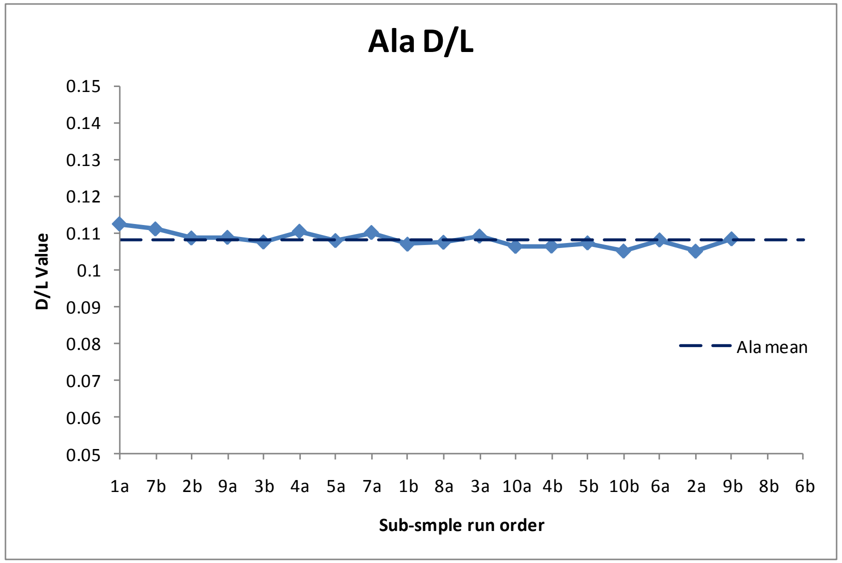
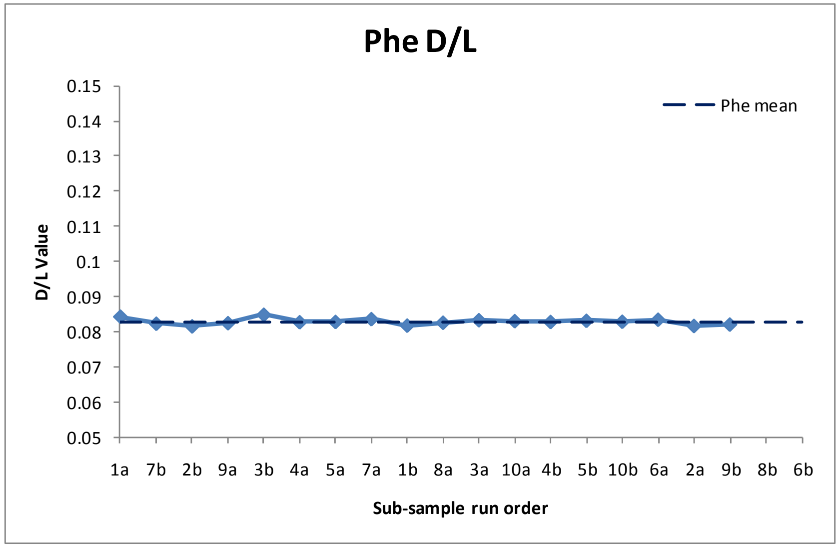
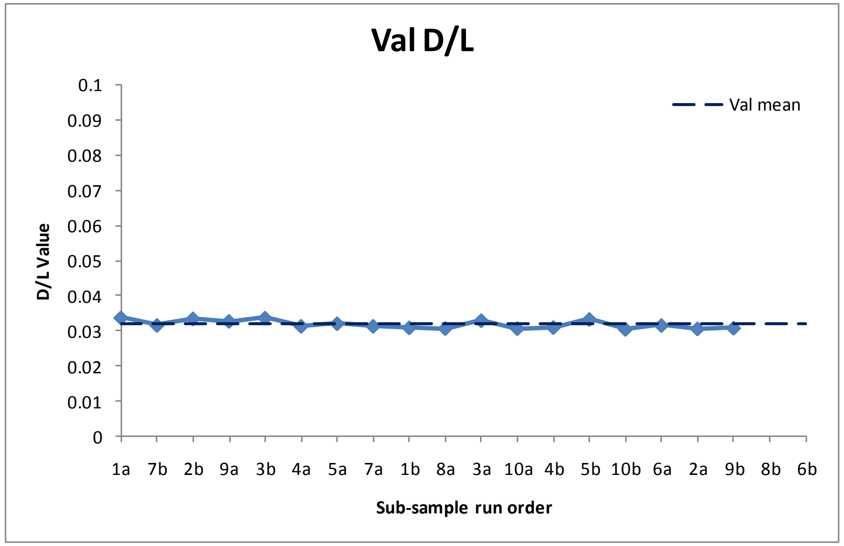


Figure 3.1: Homogeneity Amino Acid D/L Values in Analytical Sequence Order (continued).







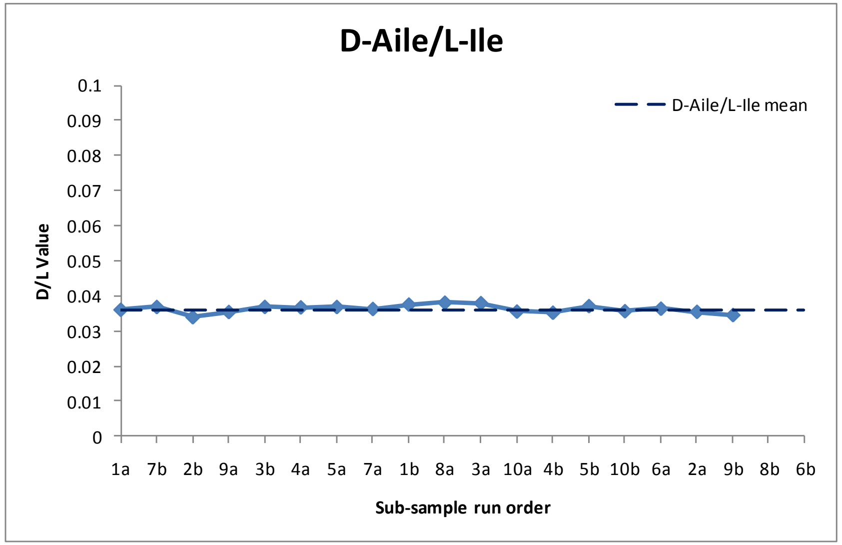


Figure 3.1: Homogeneity Amino Acid D/L Values in Analytical Sequence Order; (continued)

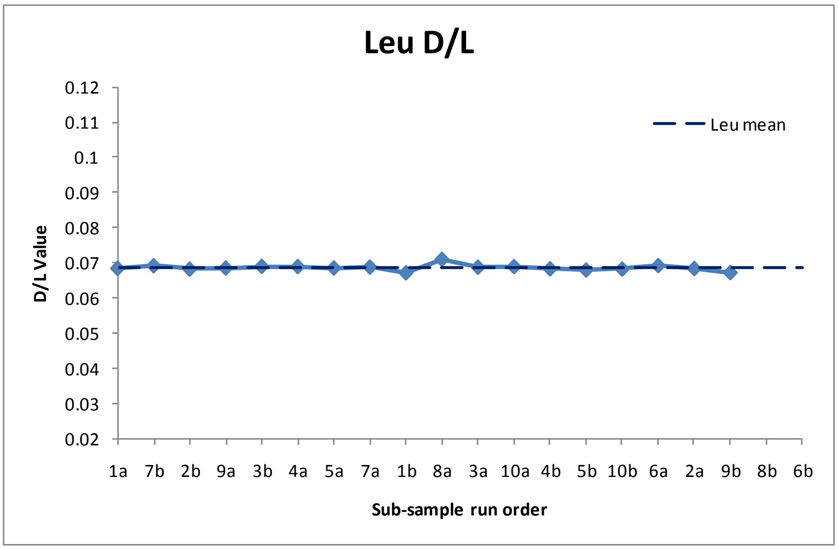
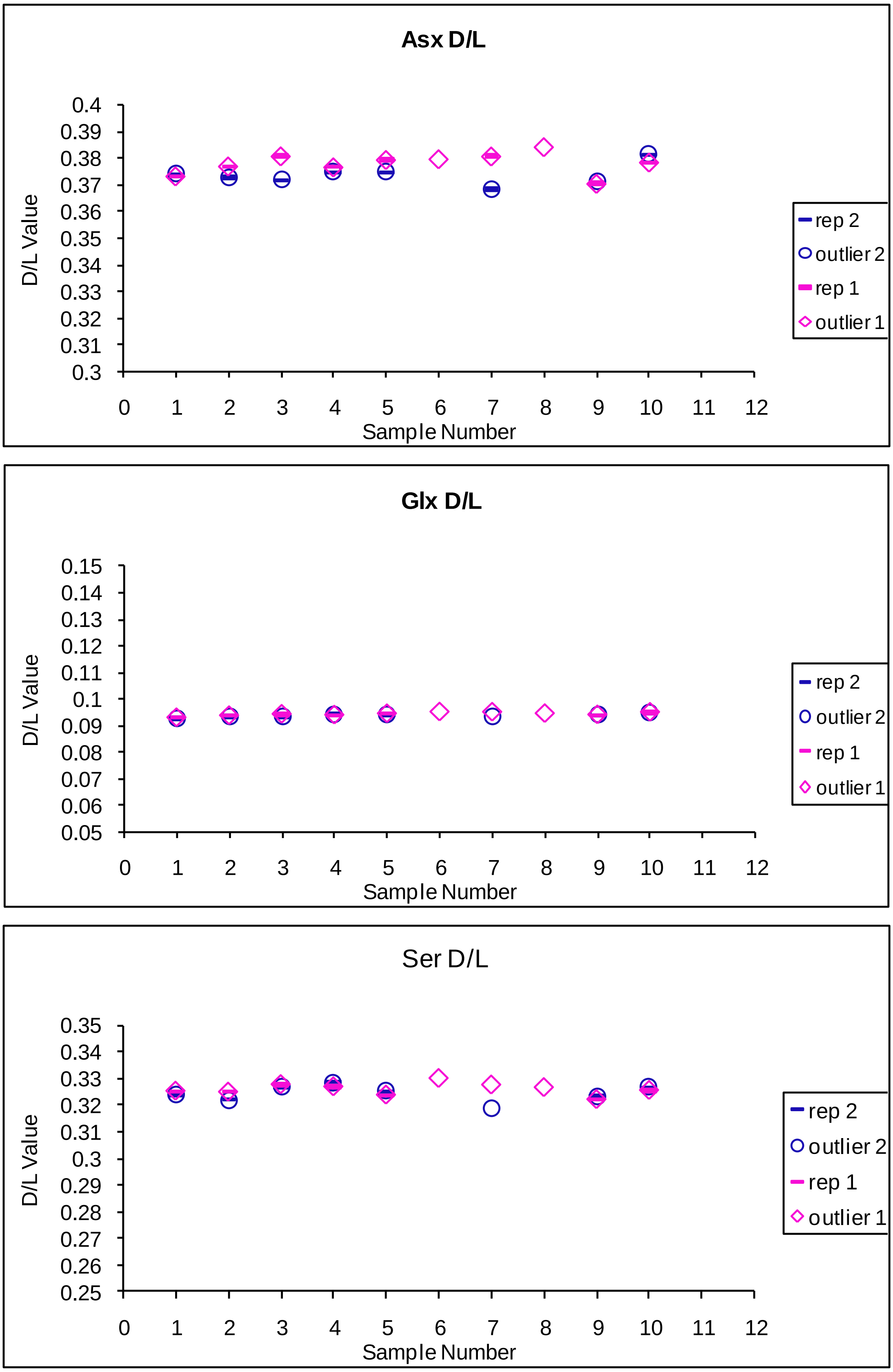


Figure 3.2: Homogeneity Amino Acid D/L Values; Paired Sub-samples showing Outliers.



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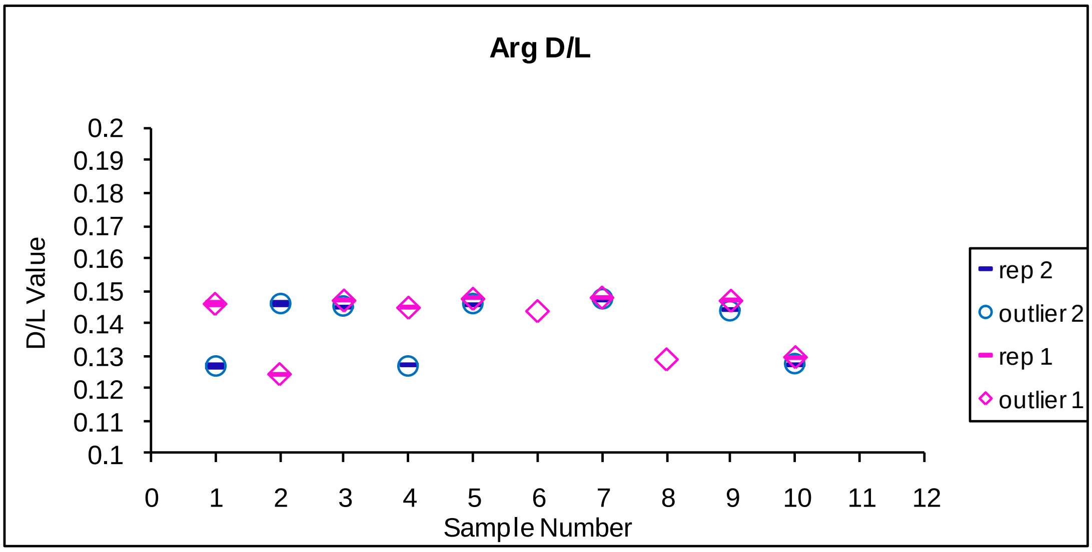

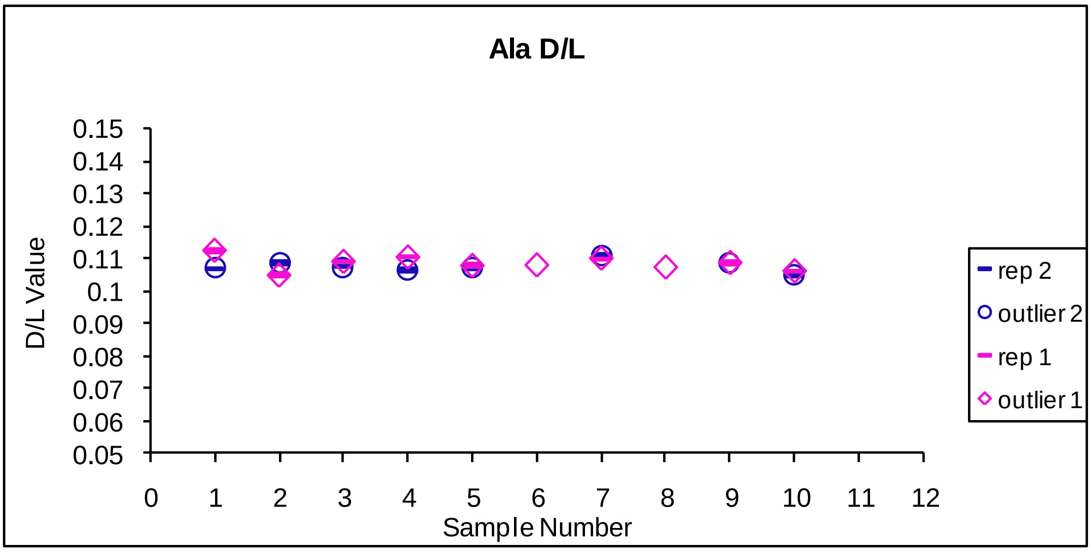

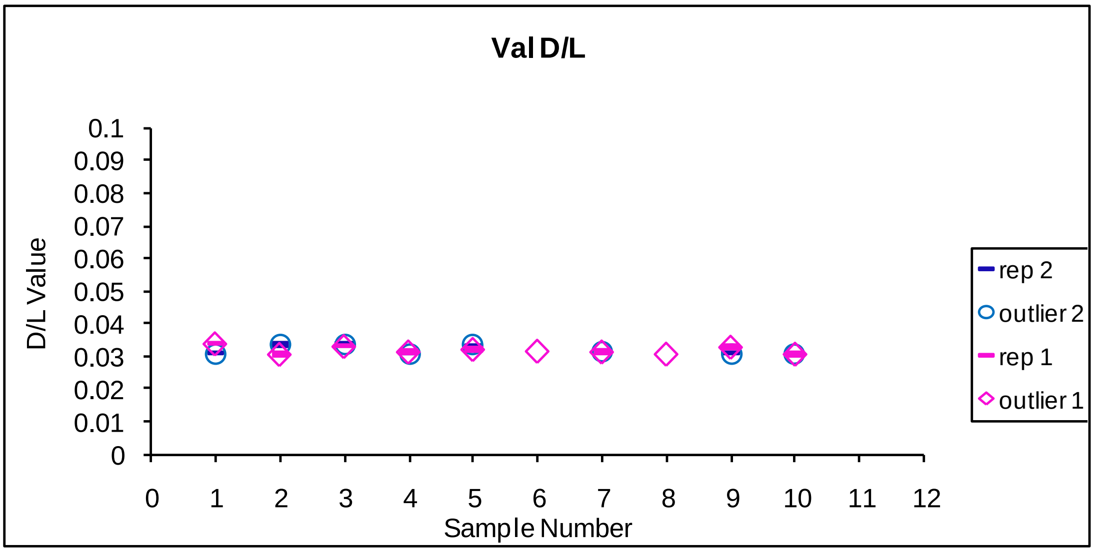

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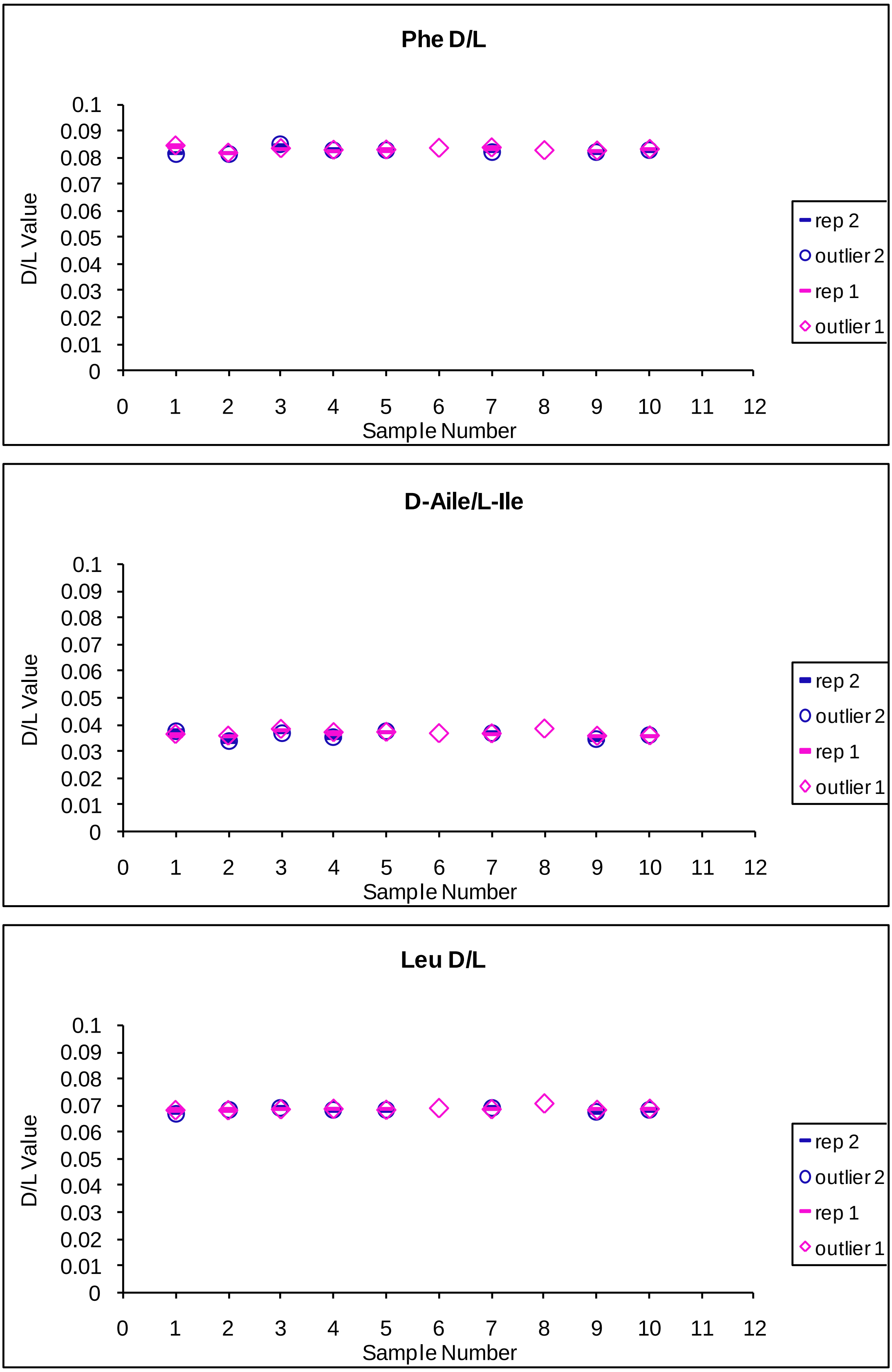


4 STATISTICAL EVALUATION; *Summary Statistics*

4.1 Precision Analysis

In keeping with the style of previously conducted inter-laboratory comparisons (Wehmiller, 1984, Wehmiller, 2010), participants were invited to submit peak information and concentration data in addition to the D/L value data requested for the proficiency study. Consequently a substantial quantity of information was captured. Due to time constraints it was not possible to evaluate all of this additional data, although a comparison of L and D amino acid concentrations would be enlightening.

Table 4.1 summarises indicative values of repeatability and reproducibility precision estimates for each amino acid derived from all participants' individual D/L values. Estimates were calculated using a one way analysis of variance (ANOVA), allowing for unequal replicate numbers. It should be noted that where **all** data have been used in the evaluation of precision estimates in Table 4.1, this includes GC D/L values derived from both peak area and height data where given, although the laboratory subsequently confirmed that in practice only peak area data would be used for chronology building. Results from the analysis of relative bias presented in Section 5, suggest possible empirical differences between methods. Therefore, all rpHPLC data and HPLC-IE data for D-alloisoleucine/L-isoleucine, have also been evaluated separately. However, because all HPLC-IE data came from the same laboratory, reproducibility (RSD_R) values should more correctly be interpreted as an intra-laboratory reproducibility or intermediate precision estimate. As GC data were submitted as average D/L values, it was not possible to determine comparable GC specific precision estimates.

The repeatability standard deviation s_r (Table 4.1), is a measure of the overall within laboratory precision derived from all participating laboratories. **On this occasion, this represents an inter-laboratory approximation of the instrumental precision only,** due to random error effects**.** This reflects the variability that a single laboratory might be expected to achieve for replicate measurements of the same sample. Typically, this may be slightly larger than instrumental precision estimates derived from a single laboratory (i.e. the $CV\%$ (or $RSD\%$) given in Tables 4.2 – 4.33) but smaller than method repeatability which includes additional variability arising from the analysis of different samples of the same material by a single laboratory, under repeatability conditions. Often the s_r is more conveniently given as the relative repeatability standard deviation expressed as a percentage, ($RSD_r\%$).

s_L is the overall inter-laboratory between sample standard deviation, and indicates the level of agreement between participants. **s_R is the inter-laboratory reproducibility standard deviation and a measure of the overall precision for any given amino acid** in the specified test material. s_R incorporates both the within and between laboratory variability and is a single measure of the variability or uncertainty of the measurement procedure associated with precision. Such determinations are more commonly used to assess data from method specific collaborative trials (Horwitz, 1995, AOAC, 2000) known as the "top-down" approach to uncertainty estimation (RSC

Analytical Methods Committee, 1995). The relative standard deviation of reproducibility ($RSD_R\%$) obtained from a collaborative trial may then be used for the assessment of proficiency test data as it provides an external value for the target standard deviation, i.e.; it describes how the data is expected to behave under conditions of best practice. However, in the absence of a collaborative trial, precision evaluation of the submitted PT results will help give an **indication** of the agreement between laboratories, albeit being slightly exaggerated due to additional method variation between participants. (Note; in the case of empirical methods, PT data should be assessed against method specific precision estimates).

All submitted results have been included in this evaluation without removal of outliers as would otherwise be the case with collaborative trail data. On this occasion it is the intention to observe the behaviour of all submitted results rather than to define best practice. It should be noted that these values have not been used in the later performance evaluation but are given for information and indicative purposes only. Further details on the calculations of S_R, S_L and S_r can be found in (ISO 5725, 1994, ISO 21748, 2010). Precision estimates are calculated using ANOVA, thus;

$$s_r = \sqrt{within\ group\ mean\ square}$$

$$s_L = \sqrt{\frac{between\ group\ mean\ square - within\ group\ mean\ square}{n}}$$

$$s_R = \sqrt{s_r^2 + s_L^2}$$

Table 4.1: Precision Estimates derived from Participants' submitted results

amino acid	no of sets of results (m)	total no of replicates (N)	mean	S_r	$RSD_r\%$	S_L	$RSD_L\%$	S_R	$RSD_R\%$
Asx D/L-all[a]	15	30	0.371	0.0038	1.02	0.0250	6.75	0.0253	6.83
Asx D/L-rpHPLC	11	26	0.364	0.0038	1.04	0.0149	4.09	0.0154	4.22
Glx D/L-all[a]	15	29	0.085	0.0041	4.79	0.0084	9.85	0.0094	10.96
Glx D/L-rpHPLC	11	25	0.085	0.0041	4.83	0.0083	9.82	0.0093	10.95
Ser D/L-rpHPLC	11	27	0.329	0.0023	0.70	0.0086	2.60	0.0089	2.69
Arg D/L-rpHPLC	9	15	0.139	0.0055	3.96	0.0188	13.52	0.0196	14.09
Ala D/L-all[a]	14	30	0.092	0.0031	3.36	0.0083	8.94	0.0088	9.55
Ala D/L-rpHPLC	11	27	0.094	0.0031	3.29	0.0059	6.29	0.0067	7.10
Val D/L-all[a]	15	31	0.028	0.0022	7.84	0.0037	13.29	0.0043	15.43
Val D/L-rpHPLC	11	27	0.029	0.0022	7.61	0.0028	9.56	0.0035	12.22
Phe D/L-all[a]	15	31	0.077	0.0034	4.44	0.0054	7.02	0.0064	8.31
Phe D/L-rpHPLC	11	27	0.077	0.0034	4.40	0.0036	4.60	0.0049	6.37
D-Aile/L-Ile -all[b]	17	36	0.035	0.0018	5.12	0.0089	25.41	0.0091	25.92
D-Aile/L-Ile -rpHPLC	11	27	0.035	0.0018	5.04	0.0103	29.22	0.0105	29.65
D-Aile/L-Ile -HPLC-IE	2	5	0.031	0.0019	6.04	-	-	0.0019	6.04
D-Aile/L-Ile -GC					Not determined				
Leu D/L-all[a]	13	28	0.060	0.0030	5.03	0.0124	20.69	0.0127	21.29
Leu D/L-rpHPLC	9	24	0.063	0.0030	4.81	0.0112	17.89	0.0116	18.52
Tyr D/L-rpHPLC	7	11	0.078	0.0008	1.07	0.0055	7.10	0.0056	7.18

[a] = rpHPLC and GC data [b] = rpHPLC, GC and HPLC-IE data

4.2 Summary Statistics

Summary statistics are presented in Tables 4.2-4.33 for rpHPLC peak areas and concentrations, peak-height values for HPLC-IE and D/L values for all participants. Individual laboratory replicate D/L values as submitted, are also shown graphically against the assigned values determined in Section 5, for comparison. It should be noted that GC data was submitted as the mean $\bar{x}$ of *n* replicates with a stated standard deviation, *s*, and these have been displayed as the mean value with associated error bars on the charts. Data are presented as submitted on the result proforma for each of the total hydrolysed amino acids, including internal standard data provided by participants. Only one laboratory reported data for the free amino acids and this has not been included in this report. Calculations have been carried out on each laboratory's results to give the instrumental precision estimate as the standard deviation (s) and relative standard deviation, $RSD\%$, also known as the coefficient of variance, $CV\%$, for each amino acid, where;

$$RSD\%\ or\ CV\% = \left({}^{s}/_{\bar{x}}\right) \times 100$$

Additionally, the experimental standard deviation (or standard error or standard uncertainty) of the mean ($u(\bar{x})$) and the relative standard uncertainty of the mean ($RSU\%$), have been determined. Each laboratory's expanded uncertainty to 2 std deviations or an approximate 95% confidence level, has been evaluated for each amino acid and data are presented in figures to illustrate the effect of uncertainty on the mean value of submitted replicate data.

4.2.1 Experimental Standard Uncertainty of the Mean $u(\bar{x})$

Depending on information sources, there are various names used to describe ($u(\bar{x})$) as mentioned above. Standard uncertainty is always expressed as a standard deviation, thus either experimental standard deviation or standard uncertainty of the mean would be acceptable. In this report, $u(\bar{x})$ will be referred to as the *experimental standard uncertainty of the mean* and reflects the confidence in the mean of replicate values, i.e.; the larger the value of *n,* the greater the confidence in the mean $\bar{x}$ as an estimate of the true value μ, and the smaller the uncertainty. **<u>Note</u>; The observed standard deviation of replicate instrumental measurements describes the distribution of data and is not the same as the uncertainty estimate for the mean**. (Strictly speaking this should be determined using independent repeated measurements and not replicate measurements of the same sample).

Thus;

Experimental standard uncertainty of the mean is obtained from; $u(\bar{x}) = {}^{s}/_{\sqrt{n}}$

Which, expressed as a percentage relative to the mean; $RSU\% = \left({}^{u(\bar{x})}/_{\bar{x}}\right) \times 100$

It is important to appreciate that $u(\bar{x})$ is the uncertainty associated with the mean of replicate instrumental results only. It **contributes** to the **bias** component of the overall combined uncertainty associated with the measurement system (see Figure 6.1) but is **only one component of the uncertainty that should be reported with the mean of analytical results**. Measurement uncertainty determination is discussed this in more detail in Section 6 later in the report.

As a standard uncertainty, $u(\bar{x})$ represents a confidence level equivalent to 68% or 1 standard deviation. This means that 68 percent of the means of repeated replicate results will fall within these limits either side of the mean determined by $\bar{x} \pm u(\bar{x})$. This gives little confidence as in nearly one out of every three occasions, the mean is likely to fall outside of this range. However, in practice it is often more helpful to consider a confidence interval equivalent to 2 standard deviations or a

95.4% probability level in experimental design (usually rounded to 95% for simplicity). This equates to a 1 in 20 chance of falling outside the range. 3 standard deviations would be equivalent to 99.7% confidence or 1 in 300.

To determine these extended limits of confidence an Expanded Uncertainty (U) is calculate thus;

$U = u(\bar{x}) \times k$ where k is the coverage factor set according to the required confidence level.

Expanded uncertainty is more usually determined following the combination of all individual standard uncertainty components as demonstrated in Section 6. However, it may also be helpful to observe the effect of uncertainty on individual elements to aid method development or quality improvements.

The coverage factor, , and its role in determining the Expanded uncertainty is now considered in more detail below.

4.2.2 Setting the correct coverage factor for Expanded Uncertainty determination.

Theoretically, if analytical results represented an entire population and the true value μ and standard deviation σ were known, it would be possible to calculate the range of values within which repeated experimental means $\bar{x}$ of *n* measurements were likely to fall with a certain level of confidence. As discussed above, for most general applications, a 2 standard deviation or approximately 95% confidence level is usually acceptable. Thus in this instance $k = 2$ (actually its 1.96σ) and the relevant confidence interval where (approx) 95% of $\bar{x}$ values would lie would be in the range;

$$\mu - \left[2 \times \frac{\sigma}{\sqrt{n}}\right] \quad to \quad \mu + \left[2 \times \frac{\sigma}{\sqrt{n}}\right]$$

However, in real terms, the true value of μ and σ cannot be known and the aim of experimental investigations is to get the best estimate of μ from the sample mean, $\bar{x}$. Where the number of replicate measurements is large, i.e.; *n*=30 or more (Currell and Dowman, 2005) then the distribution of mean values conforms with the expectation of normality. However for decreasing values of *n*, the characteristic bell shaped curve of the normal distribution flattens and widens reflecting the reduced confidence in the value $\bar{x}$ as the best estimate of μ and our uncertainty estimate increases. To compensate for the use of the sample standard deviation, *s*, rather than the population standard deviation σ, *k*=2 is replaced by the critical *t*-value as a correction term. The value of *t* depends on the value of *n* and the required level of confidence and can be read from any two-tailed *t*-table in statistical texts. Thus for *n*=5 (degrees of freedom=4) at 95% confidence level (α=0.05), *t*=3.18 compared to the original value of *k=2*, or for a pair of replicates; *n*=2, *df*=1, *t*=12.7 and the expanded uncertainty becomes over six times larger than otherwise predicted if *k*=2! Thus the range in which the true value lies with 95% confidence broadens and becomes;

$$\bar{x} - \left[t_{(2,0.05,df)} \times \frac{\sigma}{\sqrt{n}}\right] \quad to \quad \bar{x} + \left[t_{(2,0.05,df)} \times \frac{\sigma}{\sqrt{n}}\right]$$

In practice and often for simplicity rather than intent, laboratories can often be found to overlook this *t*-value correction by quoting expanded uncertainties derived from the more favorable *k*=2.

Relative Expanded uncertainties of the submitted results using both $k=t_{(0.05,df)}$ and the more frequently used *k*=2 have been calculated and values expressed as a percentage. For each amino acid, data are given in tables and presented as two comparative figures. Note that where a single replicate value is reported, no uncertainty estimation can be made.

The differences observed in expanded uncertainties between different amino acids for a single laboratory highlights the ease or difficulty of analysis and instrument repeatability. A comparison of expanded uncertainties across all laboratories for any individual amino acid also demonstrates the effect of different methods or even using different numbers of replicates for the same method.

Whilst these effects are interesting to observe analytically, the effect of the number of replicates is an important practical consideration. Demands for quality and lower uncertainty estimates must be balanced against the extra cost and time incurred by increasing replicate numbers not to mention material availability and often it is financial and resource constraints that become deciding factors.

4.3 t-Distribution vs Normal Distribution

The relationship between the t-distribution and the Normal or Gaussian distribution at 2 standard deviations (95% confidence) is shown below in Figure 4.1. It illustrates the t-distribution deviation (red line) away from normal (black line) for low sample numbers, (degrees of freedom (n-1) between 1 - 35 where n is the sample size). The t-value given on the y-axis is used as the correction term in the calculation of expanded uncertainty. t-values are given in Appendix 3.

It can be clearly seen that for a pair if replicate values; (df = 1), there is a significant deviation from normal, introducing a correction factor more than 10x larger (t-value = 12.7) on the standard uncertainty estimate. Increasing the number of replicate values to n =3 (df = 2), reduces the t-value correction to 4.3, and for n = 4 (df = 3), the t-value correction becomes 3.2. Thus the effect of increasing the number of replicate values from 2 to 3 will make a substantial reduction in the expanded uncertainty estimate, whilst increasing the number of replicates from 3 to 4 will still make an improvement, but the difference will not be quite as significant. The level of benefit gained by increasing the numbers of replicates gradually diminishes until normality is achieved at about n = 25.

The contribution of a particular standard uncertainty estimate to the overall uncertainty budget, should also be borne in mind. For example; the contribution of instrumental analytical precision is likely to me much smaller than the contribution from method precision between different samples. It therefore makes more sense to put time into increasing the number or individual samples tested than spending the same time increasing the number of instrumental replicates, as there is more to gain in reducing the expanded uncertainty.

Figure 4.1: Relationship between the t-distribution and the Normal distribution at a 95% Confidence Level, for low values of *n* (degrees of freedom (*n-1*) between 1-35).

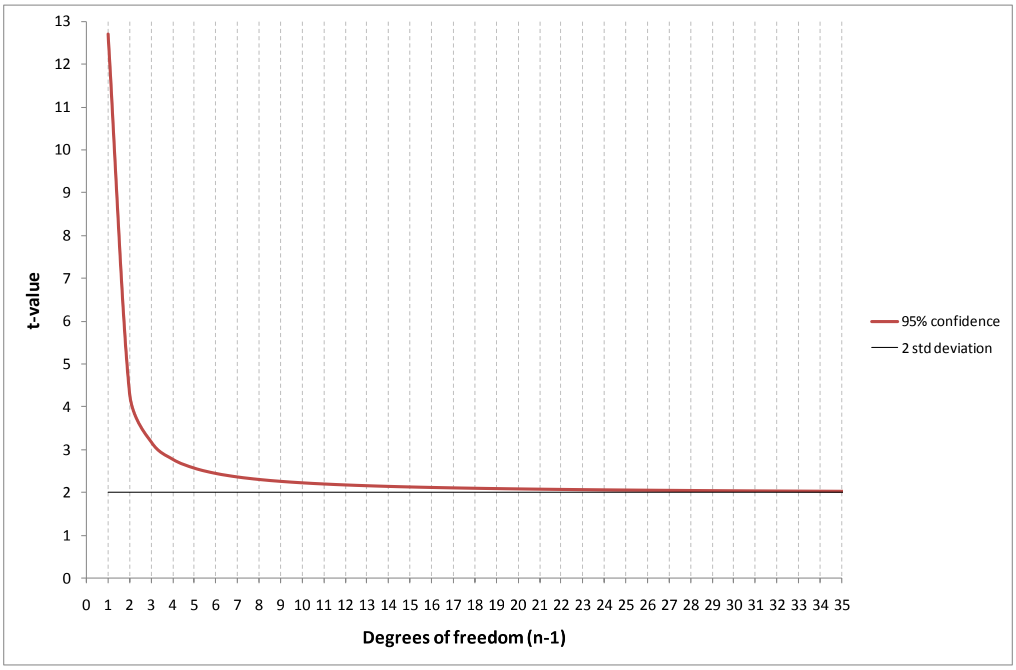


Table 4.2: Summary Statistics for L and D **Aspartic Acid / Asparagine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Asx peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	73591	75250	79183		20224	22265	23416	24780	27604	29583	41766	9	25866.6	61.93	8622.2	20.64	41.29	2.306	47.61
2	RP	9378	9423									9401	2	32.3	0.34	22.9	0.24	0.49	12.710	3.09
3	RP	8066										8066	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	23345	23254									23300	2	64.1	0.28	45.3	0.19	0.39	12.710	2.47
9	RP	20893	21494									21194	2	424.8	2.00	300.4	1.42	2.83	12.710	18.01
10	RP	12266	13209									12738	2	667.0	5.24	471.6	3.70	7.41	12.710	47.06
11	RP	8781	8677									8729	2	73.8	0.85	52.2	0.60	1.20	12.710	7.60
12	RP	10791	10919									10855	2	90.4	0.83	63.9	0.59	1.18	12.710	7.49
13	RP	7697										7697	1							
14	RP	5858										5858	1							
15	RP	4561	4647									4604	2	61.0	1.32	43.1	0.94	1.87	12.710	11.90
D-Asx peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	26238	26828	28238	30804	7011	7708	8121	8576	9578	10244	16334	10	10170.8	62.27	3216.3	19.69	39.38	2.262	44.54
2	RP	3282	3281									3282	2	0.7	0.02	0.5	0.02	0.03	12.710	0.20
3	RP	2944										2944	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	8399	8390									8395	2	6.3	0.07	4.4	0.05	0.11	12.710	0.67
9	RP	7931	8164									8048	2	164.8	2.05	116.5	1.45	2.90	12.710	18.40
10	RP	4689	5041									4865	2	248.6	5.11	175.8	3.61	7.22	12.710	45.91
11	RP	3330	3282									3306	2	33.3	1.01	23.5	0.71	1.42	12.710	9.05
12	RP	4144	4205									4175	2	43.3	1.04	30.6	0.73	1.47	12.710	9.33
13	RP	2840										2840	1							
14	RP	2211										2211	1							
15	RP	1678	1729									1703	2	36.1	2.12	25.6	1.50	3.00	12.710	19.07

Table 4.3: Summary Statistics for L and D **Aspartic Acid / Asparagine** Concentration Data (pM)

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Asx Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% (k=t_{crit})**
1	RP	3865	3910	3624		3966	4088	4022	4046	3947	4007	3942	9	137.6	3.49	45.9	1.16	2.33	2.306	2.68
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	3834	3803									3818	2	21.9	0.57	15.5	0.41	0.81	12.710	5.16
9	RP	3658	3912									3785	2	179.5	4.74	126.9	3.35	6.71	12.710	42.62
10	RP	4086	4186									4136	2	70.7	1.71	50.0	1.21	2.42	12.710	15.35
11	RP	3557	3593									3575	2	25.1	0.70	17.7	0.50	0.99	12.710	6.30
12	RP	5314	5303									5308	2	8.1	0.15	5.7	0.11	0.22	12.710	1.37
13	RP	5543										5543	1							
14	RP	6398										6398	1							
15	RP	5175	5294									5235	2	84.2	1.61	59.6	1.14	2.28	12.710	14.46
D-Asx Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% (k=t_{crit})**
1	RP	1378	1394	1293	1337	1375	1415	1395	1400	1369	1387	1374	10	35.6	2.59	11.3	0.82	1.64	2.262	1.85
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	1379	1372									1376	2	5.1	0.37	3.6	0.26	0.53	12.710	3.36
9	RP	1389	1486									1437	2	68.8	4.79	48.6	3.38	6.77	12.710	43.01
10	RP	1562	1597									1580	2	25.0	1.58	17.7	1.12	2.24	12.710	14.21
11	RP	1349	1359									1354	2	7.3	0.54	5.2	0.38	0.76	12.710	4.85
12	RP	2041	2042									2041	2	1.1	0.05	0.8	0.04	0.07	12.710	0.47
13	RP	2045										2045	1							
14	RP	2414										2414	1							
15	RP	1903	1969									1936	2	46.6	2.41	33.0	1.70	3.40	12.710	21.63

Table 4.4: Summary Statistics for L and D **Aspartic Acid / Asparagine** D/L Ratio Value

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Asx		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	0.357	0.357	0.357		0.347	0.346	0.347	0.346	0.347	0.346	0.350	9	0.0050	1.44	0.0017	0.48	0.96	2.306	1.11
2	RP	0.350	0.348									0.349	2	0.0013	0.37	0.0009	0.26	0.52	12.710	3.29
3	RP	0.365										0.365	1							
4	IE																			
5	IE																			
6.1[1]	GC_A	0.379										0.379	4	0.0190	5.01	0.0095	2.51	5.01	3.182	7.98
6.2[1]	GC_H	0.453										0.453	2	0.0005	0.11	0.0004	0.08	0.16	12.710	0.99
7.1[1]	GC_A	0.393										0.393	1							
7.2[1]	GC_H	0.427										0.427	1							
8	RP	0.360	0.361									0.361	2	0.0007	0.20	0.0005	0.14	0.28	12.710	1.76
9	RP	0.380	0.380									0.380	2	0.0002	0.04	0.0001	0.03	0.06	12.710	0.39
10	RP	0.382	0.382									0.382	2	0.0005	0.13	0.0003	0.09	0.18	12.710	1.15
11	RP	0.379	0.378									0.379	2	0.0006	0.16	0.0004	0.11	0.23	12.710	1.45
12	RP	0.384	0.385									0.385	2	0.0008	0.21	0.0006	0.14	0.29	12.710	1.84
13	RP	0.369										0.369	1							
14	RP	0.377										0.377	1							
15	RP	0.368	0.372									0.370	2	0.0030	0.80	0.0021	0.56	1.13	12.710	7.17

[1] = submitted as the mean and standard deviation of n results.

GC_A = derived using peak area

GC_H = derived using peak height

Figure 4.2: Distribution of D/L Values submitted for **Aspartic Acid / Asparagine**

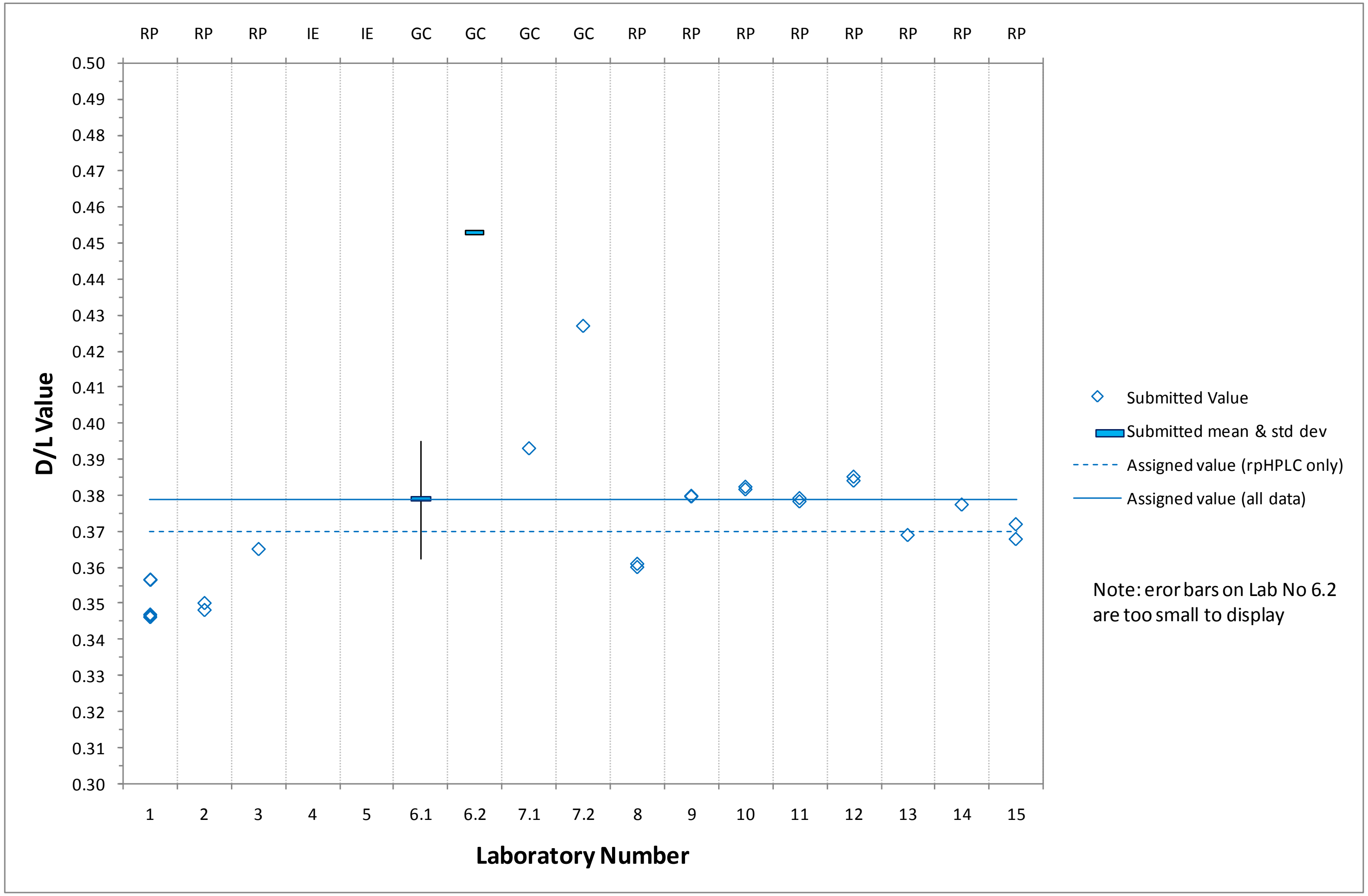


Figure 4.3: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Aspartic Acid / Asparagine** (value of n displayed).

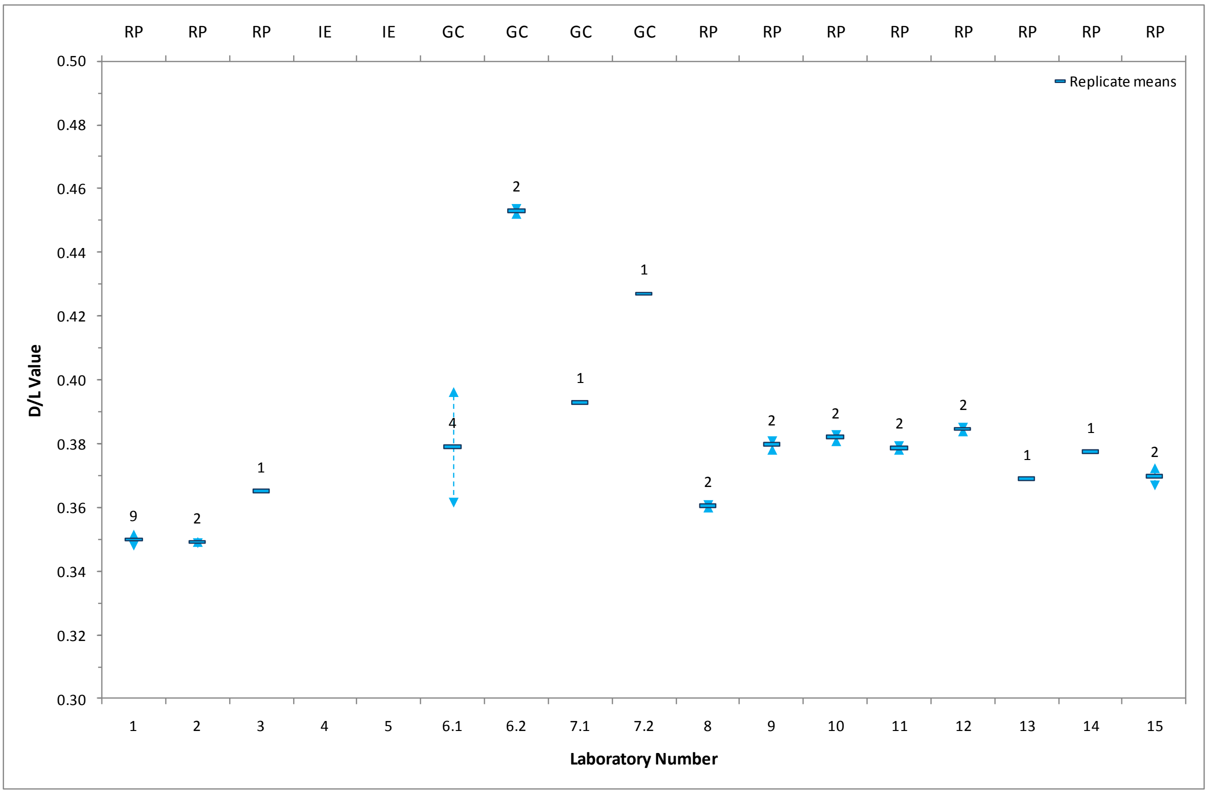


Figure 4.4: Experimental Expanded Uncertainty **(k=$t_{(0.05,df)}$)** of the Mean D/L value for **Aspartic Acid / Asparagine** (value of n displayed).

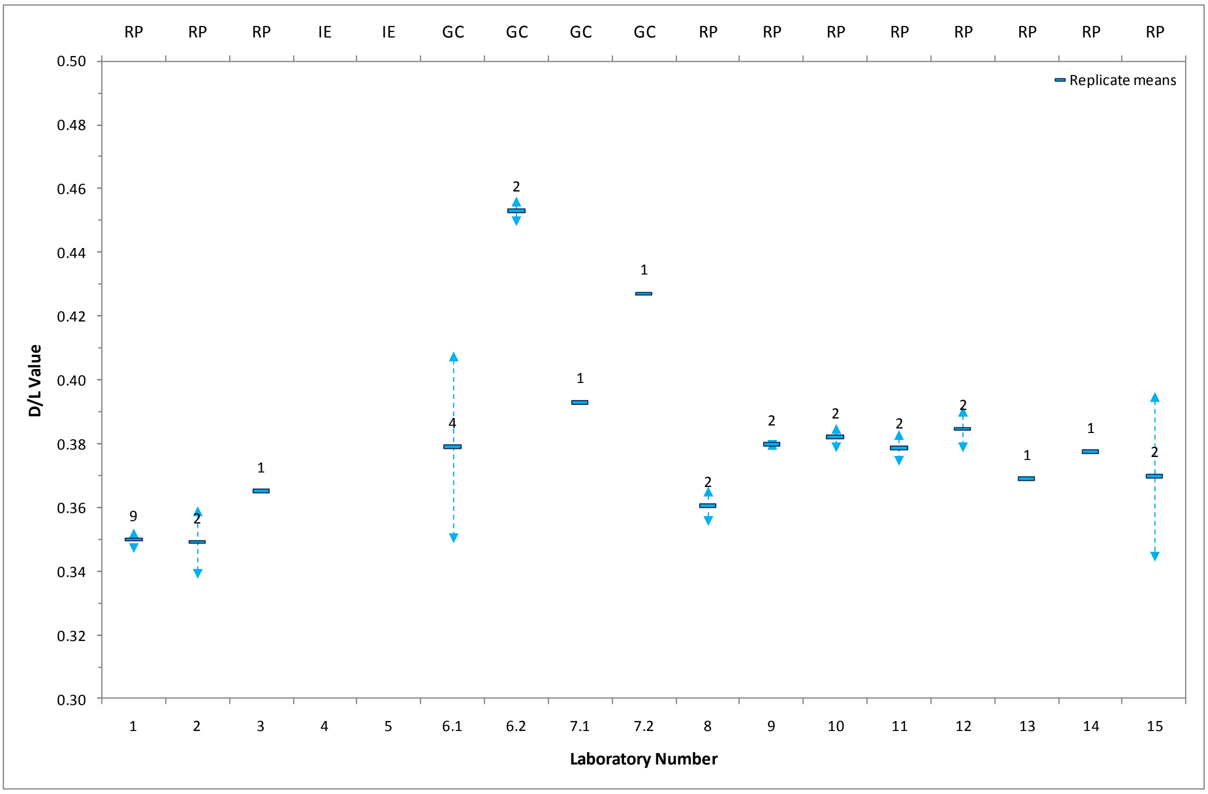


Table 4.5: Summary Statistics for L and D **Glutamic Acid / Glutamine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Glx peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	82179	98798			27115	29758	31288	33115	37082	39796	47391	8	27260.5	57.52	9638.1	20.34	40.67	2.365	48.09
2	RP	12181	12147									12164	2	24.4	0.20	17.2	0.14	0.28	12.710	1.80
3	RP	10638										10638	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	31099	30960									31029	2	98.3	0.32	69.5	0.22	0.45	12.710	2.85
9	RP	26640	27098									26869	2	323.7	1.20	228.9	0.85	1.70	12.710	10.83
10	RP	15881	16706									16294	2	582.8	3.58	412.1	2.53	5.06	12.710	32.15
11	RP	11462	11281									11372	2	128.1	1.13	90.6	0.80	1.59	12.710	10.12
12	RP	13634	13878									13756	2	173.0	1.26	122.3	0.89	1.78	12.710	11.30
13	RP	10032										10032	1							
14	RP	7552										7552	1							
15	RP	5776	5898									5837	2	86.6	1.48	61.3	1.05	2.10	12.710	13.34
D-Glx peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	7591	7771	8562	9233	2054	2264	2377	2510	2811	3016	4819	10	3030.3	62.88	958.3	19.89	39.77	2.262	44.99
2	RP	844	847									845	2	2.2	0.26	1.5	0.18	0.36	12.710	2.31
3	RP	802										802	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	2634	2633									2633	2	0.8	0.03	0.5	0.02	0.04	12.710	0.26
9	RP	2533	2566									2549	2	23.0	0.90	16.3	0.64	1.28	12.710	8.11
10	RP	1493	1576									1535	2	58.6	3.82	41.4	2.70	5.40	12.710	34.32
11	RP	1047	1038									1043	2	6.5	0.62	4.6	0.44	0.88	12.710	5.57
12	RP	1287	1312									1300	2	18.3	1.41	12.9	0.99	1.99	12.710	12.64
13	RP	872										872	1							
14	RP	690										690	1							
15	RP	513	519									516	2	4.3	0.82	3.0	0.58	1.17	12.710	7.41

Table 4.6: Summary Statistics for L and D **Glutamic Acid / Glutamine** Concentration Data (pM)

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Glx Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	4316	5133			5317	5464	5375	5407	5302	5390	5213	8	375.9	7.21	132.9	2.55	5.10	2.365	6.03
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	5107	5063									5085	2	31.3	0.62	22.1	0.44	0.87	12.710	5.53
9	RP	4870	5149									5009	2	197.6	3.94	139.7	2.79	5.58	12.710	35.44
10	RP	5523	5527									5525	2	2.6	0.05	1.9	0.03	0.07	12.710	0.43
11	RP	4848	4877									4862	2	20.4	0.42	14.4	0.30	0.59	12.710	3.78
12	RP	7009	7036									7023	2	19.1	0.27	13.5	0.19	0.38	12.710	2.44
13	RP	7542										7542	1							
14	RP	8611										8611	1							
15	RP	6842	7015									6928	2	122.6	1.77	86.7	1.25	2.50	12.710	15.90
D-Glx Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	399	404	392	401	403	416	408	410	402	408	404	10	6.7	1.65	2.1	0.52	1.04	2.262	1.18
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	433	431									432	2	1.4	0.33	1.0	0.23	0.46	12.710	2.95
9	RP	463	488									475	2	17.3	3.64	12.2	2.57	5.15	12.710	32.73
10	RP	519	522									520	2	1.5	0.29	1.1	0.20	0.41	12.710	2.60
11	RP	443	449									446	2	4.1	0.93	2.9	0.66	1.31	12.710	8.33
12	RP	661	665									663	2	2.8	0.42	2.0	0.30	0.60	12.710	3.79
13	RP	655										655	1							
14	RP	787										787	1							
15	RP	608	618									613	2	6.8	1.11	4.8	0.78	1.57	12.710	9.97

Table 4.7: Summary Statistics for L and D **Glutamic Acid / Glutamine** D/L Ratio Value

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Glx		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	0.092	0.079			0.076	0.076	0.076	0.076	0.076	0.076	0.078	8	0.0058	7.38	0.0020	2.61	5.22	2.365	6.17
2	RP	0.069	0.070									0.069	2	0.0003	0.46	0.0002	0.32	0.65	12.710	4.11
3	RP	0.075										0.075	1							
4	IE																			
5	IE																			
6.1[1]	GC_A	0.082										0.082	4	0.0130	15.85	0.0065	7.93	15.85	3.182	25.23
6.2[1]	GC_H	0.105										0.105	1							
7.1[1]	GC_A	0.086										0.086	1							
7.2[1]	GC_H	0.086										0.086	1							
8	RP	0.085	0.085									0.085	2	0.0000	0.00	0.0000	0.00	0.00	12.710	0.00
9	RP	0.095	0.095									0.095	2	0.0003	0.30	0.0002	0.21	0.43	12.710	2.72
10	RP	0.094	0.094									0.094	2	0.0002	0.24	0.0002	0.17	0.34	12.710	2.17
11	RP	0.091	0.092									0.092	2	0.0005	0.51	0.0003	0.36	0.72	12.710	4.55
12	RP	0.094	0.095									0.094	2	0.0001	0.15	0.0001	0.11	0.21	12.710	1.34
13	RP	0.087										0.087	1							
14	RP	0.091										0.091	1							
15	RP	0.089	0.088									0.088	2	0.0006	0.66	0.0004	0.47	0.93	12.710	5.93

[1]= submitted as the mean and standard deviation of n results.

GC_A = derived using peak area

GC_H = derived using peak height

Figure 4.5: Distribution of D/L Values submitted for **Glutamic Acid / Glutamine**

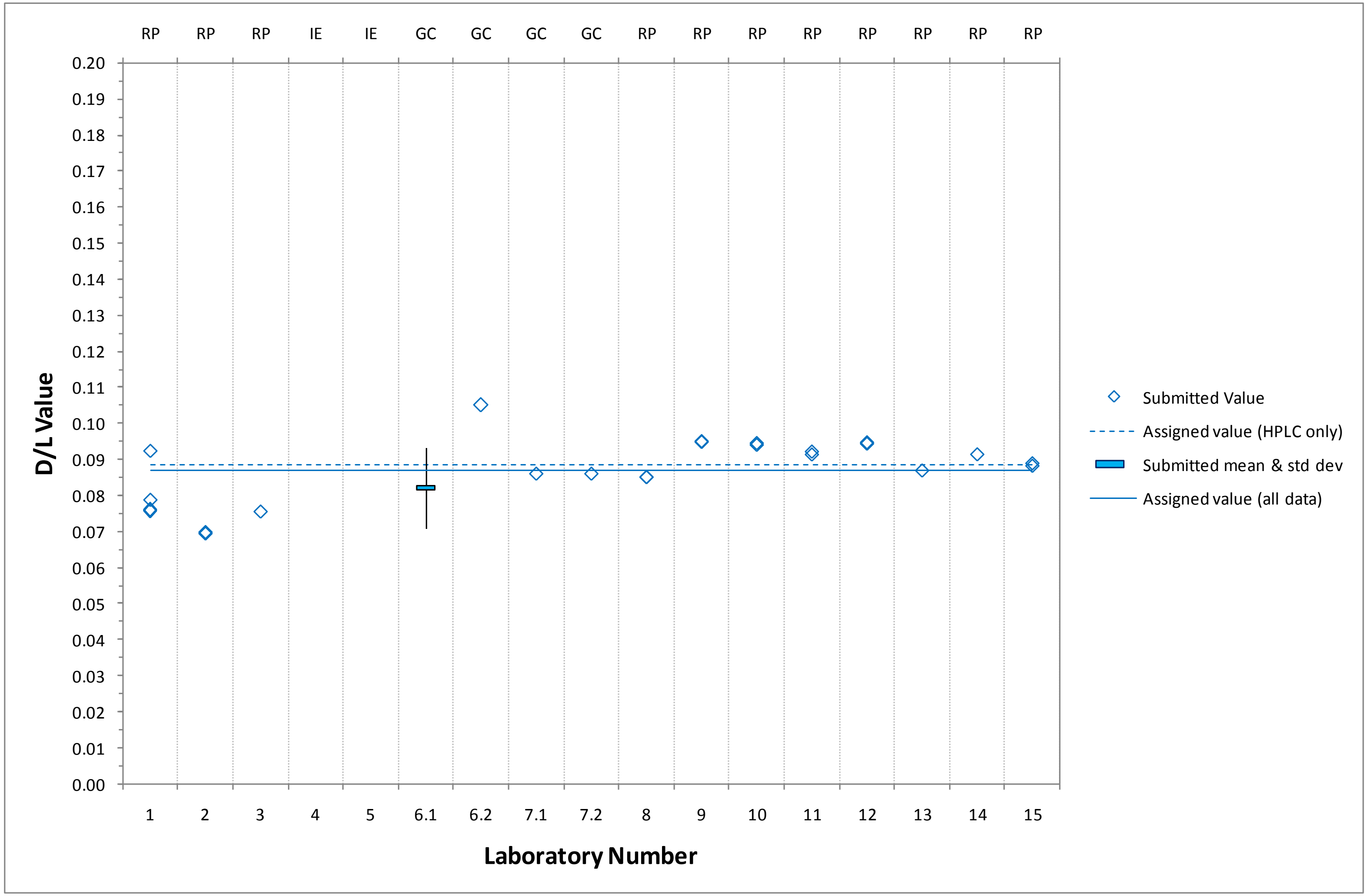


Figure 4.6: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Glutamic Acid / Glutamine** (value of n displayed).

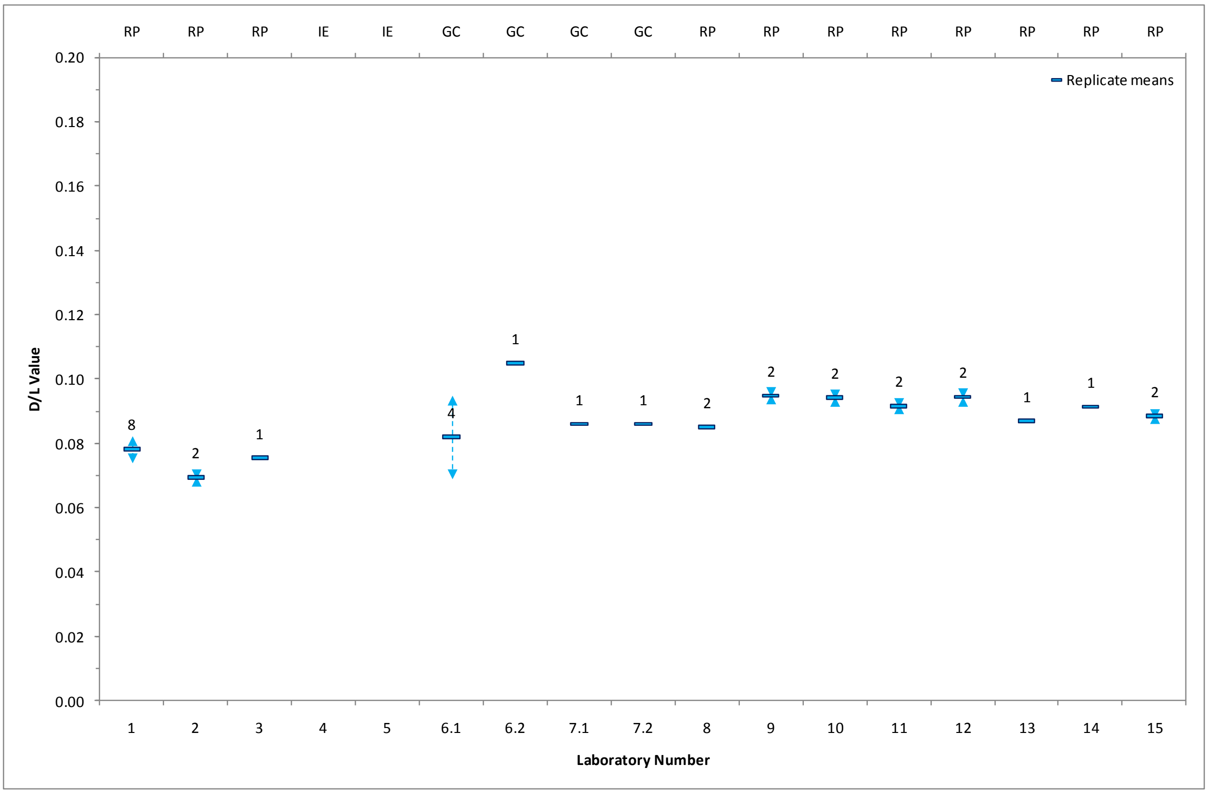


Figure 4.7: Experimental Expanded Uncertainty **(k=$t_{(0.05,df)}$)** of the Mean D/L value for **Glutamic Acid / Glutamine** (value of n displayed).

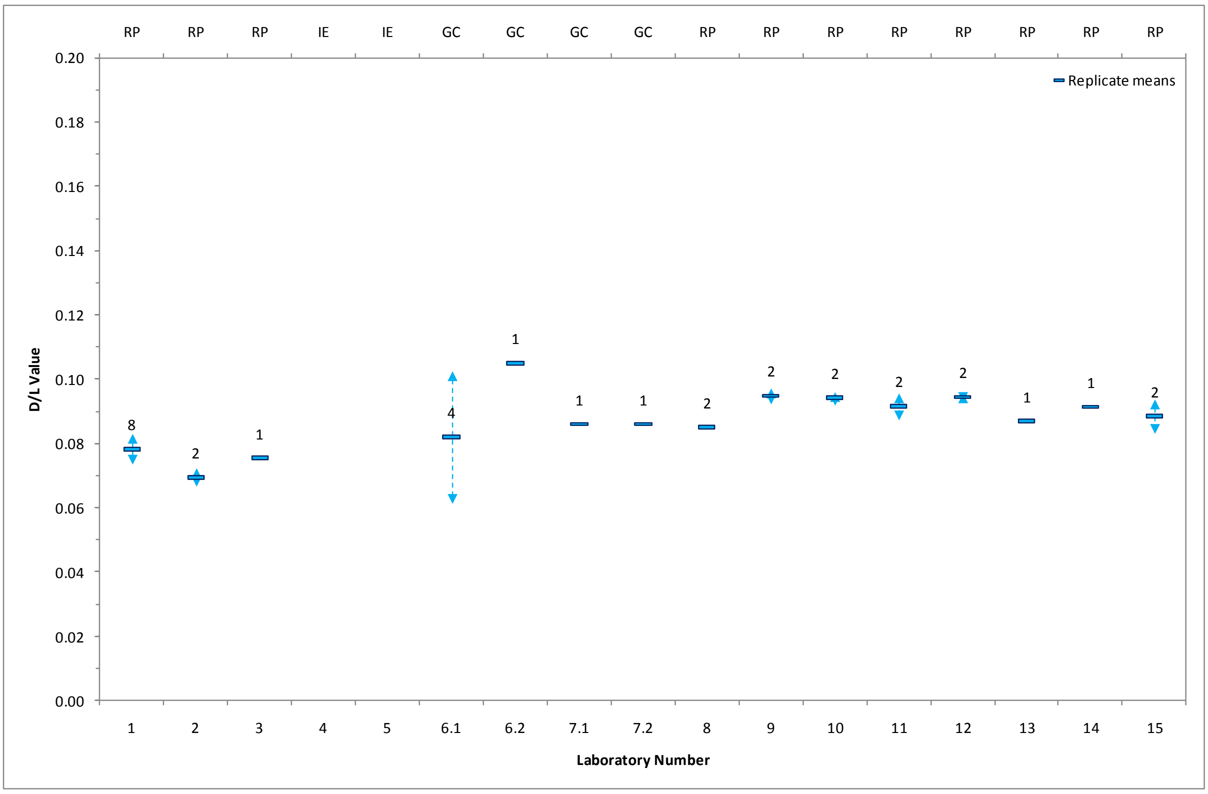


Table 4.8: Summary Statistics for L and D **Serine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Ser peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	44504	45521	49970	53346	12304	13513	14226	14912	16725	17961	26498	10	17476.2	65.95	5526.5	20.86	41.71	2.262	47.18
2	RP	5501	5498									5499	2	2.3	0.04	1.6	0.03	0.06	12.710	0.38
3	RP	4558										4558	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	13686	13645									13666	2	28.4	0.21	20.1	0.15	0.29	12.710	1.87
9	RP	12239	12575									12407	2	238.0	1.92	168.3	1.36	2.71	12.710	17.24
10	RP	7090	7640									7365	2	389.1	5.28	275.2	3.74	7.47	12.710	47.49
11	RP	5180	5100									5140	2	56.7	1.10	40.1	0.78	1.56	12.710	9.91
12	RP	6331	6455									6393	2	87.9	1.38	62.2	0.97	1.95	12.710	12.36
13	RP	4607										4607	1							
14	RP	3465										3465	1							
15	RP	2642	2720									2681	2	54.8	2.04	38.7	1.44	2.89	12.710	18.37
D-Ser peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**			
1	RP	14661	14999	16524	17705	4029	4455	4650	4892	5469	5839	8729	10	5802.4	66.47	1834.9	21.02	42.04	2.262	47.55
2	RP	1766	1815									1790	2	34.9	1.95	24.7	1.38	2.76	12.710	17.52
3	RP	1623										1623	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	4713	4724									4718	2	7.5	0.16	5.3	0.11	0.23	12.710	1.43
9	RP	3976	4110									4043	2	95.1	2.35	67.3	1.66	3.33	12.710	21.15
10	RP	2358	2536									2447	2	125.8	5.14	88.9	3.63	7.27	12.710	46.19
11	RP	1681	1659									1670	2	15.6	0.93	11.0	0.66	1.32	12.710	8.40
12	RP	2085	2125									2105	2	28.9	1.37	20.4	0.97	1.94	12.710	12.34
13	RP	1471										1471	1							
14	RP	1131										1131	1							
15	RP	838	868									853	2	21.2	2.49	15.0	1.76	3.51	12.710	22.34

Table 4.9: Summary Statistics for L and D **Serine** Concentration Data (pM)

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Ser Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	2337	2365	2287	2316	2413	2481	2444	2435	2391	2433	2390	10	62.4	2.61	19.7	0.82	1.65	2.262	1.87
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	2247	2231									2239	2	11.4	0.51	8.0	0.36	0.72	12.710	4.56
9	RP	2224	2375									2300	2	107.1	4.66	75.7	3.29	6.59	12.710	41.85
10	RP	2451	2513									2482	2	43.6	1.76	30.8	1.24	2.48	12.710	15.78
11	RP	2178	2192									2185	2	9.7	0.44	6.9	0.31	0.63	12.710	3.99
12	RP	3236	3254									3245	2	12.7	0.39	9.0	0.28	0.55	12.710	3.51
13	RP	3443										3443	1							
14	RP	3927										3927	1							
15	RP	3112	3216									3164	2	73.7	2.33	52.1	1.65	3.29	12.710	20.92
D-Ser Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	770	779	756	769	790	818	799	799	782	791	785	10	17.9	2.28	5.7	0.72	1.44	2.262	1.63
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	774	772									773	2	1.1	0.14	0.8	0.10	0.20	12.710	1.25
9	RP	723	776									749	2	38.2	5.09	27.0	3.60	7.20	12.710	45.76
10	RP	815	834									825	2	13.3	1.61	9.4	1.14	2.28	12.710	14.49
11	RP	707	713									710	2	4.3	0.61	3.1	0.43	0.87	12.710	5.50
12	RP	1065	1071									1068	2	4.1	0.39	2.9	0.27	0.55	12.710	3.49
13	RP	1100										1100	1							
14	RP	1282										1282	1							
15	RP	987	1026									1007	2	27.9	2.77	19.7	1.96	3.92	12.710	24.89

Table 4.10: Summary Statistics for L and D **Serine** D/L Ratio Value

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Serine		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	0.329	0.330	0.331	0.332	0.327	0.330	0.327	0.328	0.327	0.325	0.329	10	0.0020	0.62	0.0006	0.20	0.39	2.262	0.44
2	RP	0.321	0.330									0.326	2	0.0065	1.99	0.0046	1.41	2.82	12.710	17.89
3	RP	0.356										0.356	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	0.344	0.346									0.345	2	0.0014	0.41	0.0010	0.29	0.58	12.710	3.68
9	RP	0.325	0.327									0.326	2	0.0014	0.44	0.0010	0.31	0.62	12.710	3.91
10	RP	0.333	0.332									0.332	2	0.0005	0.14	0.0003	0.10	0.20	12.710	1.30
11	RP	0.324	0.325									0.325	2	0.0005	0.17	0.0004	0.12	0.24	12.710	1.52
12	RP	0.329	0.329									0.329	2	0.0000	0.00	0.0000	0.00	0.00	12.710	0.02
13	RP	0.319										0.319	1							
14	RP	0.326										0.326	1							
15	RP	0.317	0.319									0.318	2	0.0014	0.44	0.0010	0.31	0.62	12.710	3.97

Figure 4.8: Distribution of D/L Values submitted for **Serine**

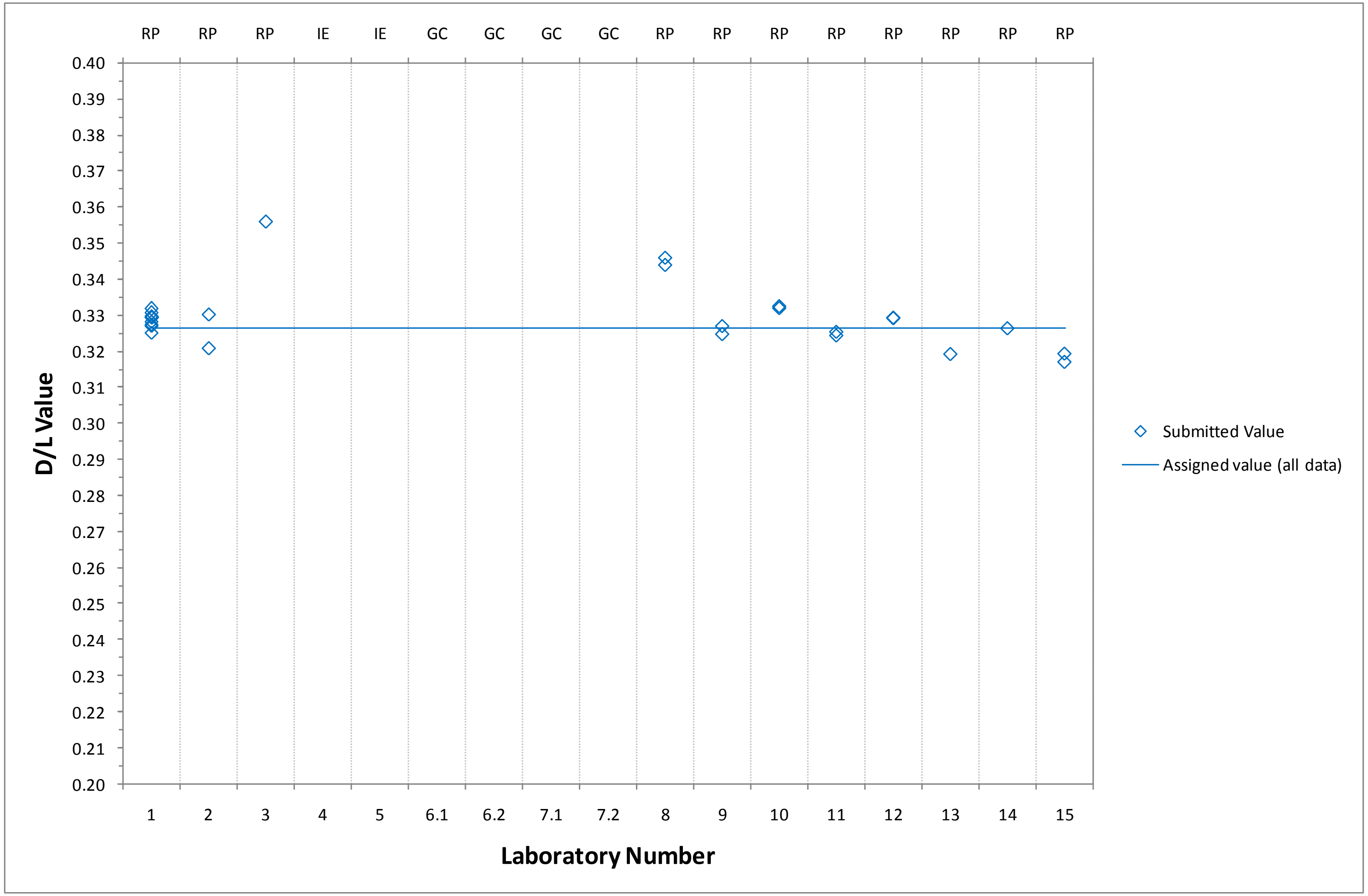


Figure 4.9: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Serine** (value of n displayed).

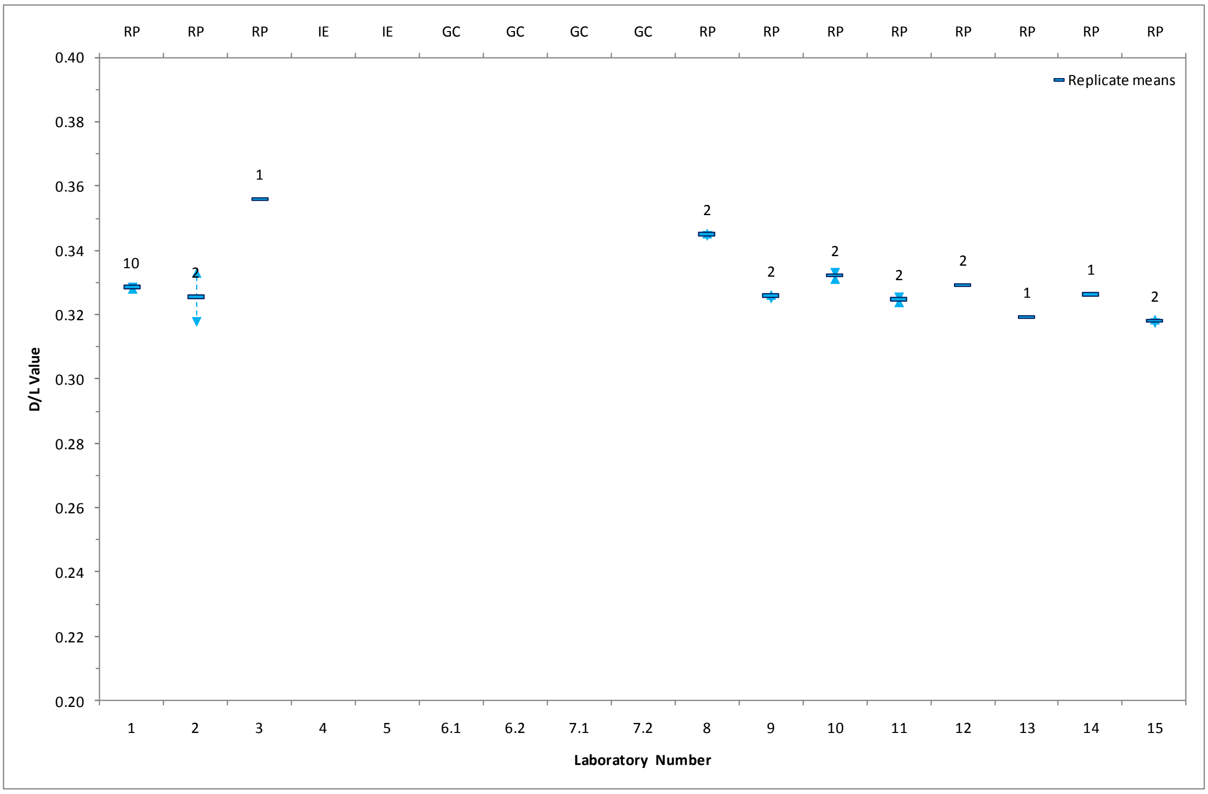


Figure 4.10: Experimental Expanded Uncertainty **(k=$t_{(0.05,df)}$)** of the Mean D/L value for **Serine** (value of n displayed).

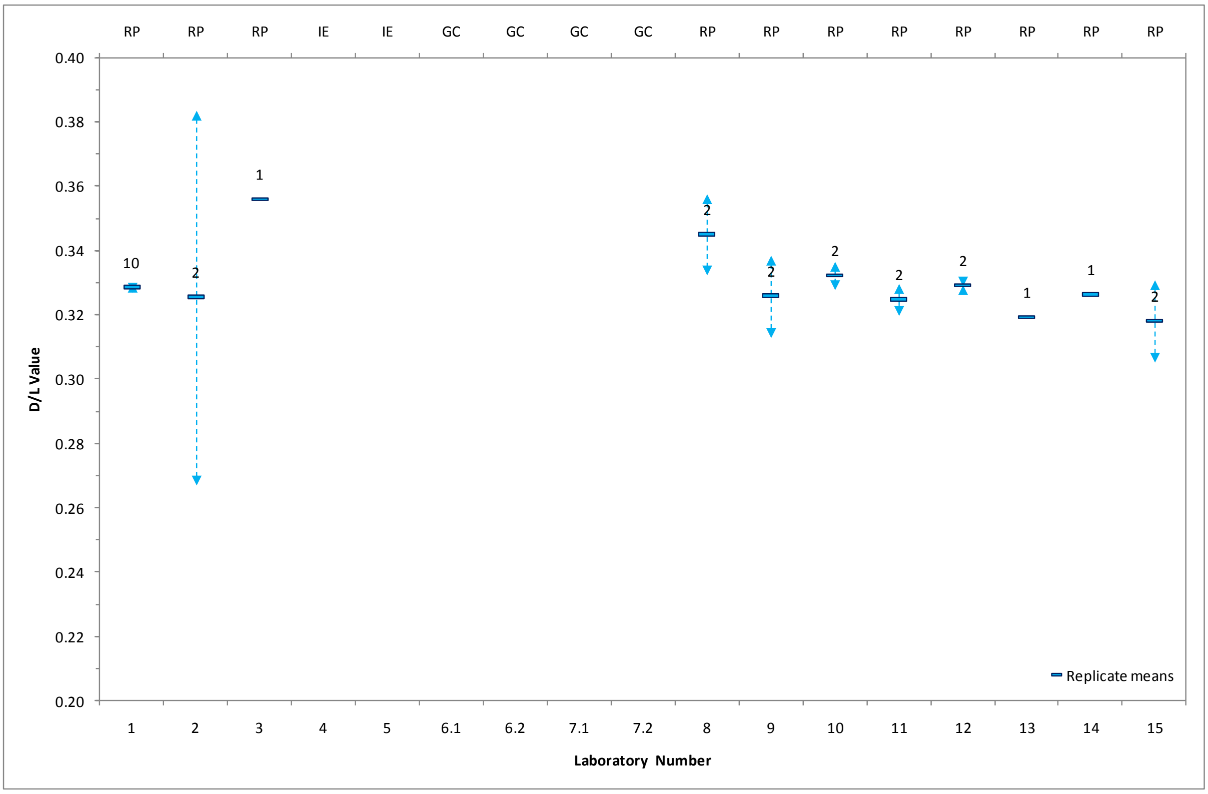


Table 4.11: Summary Statistics for L and D **Arginine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Arg peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP																			
2	RP	6367	6282									6324	2	60.5	0.96	42.8	0.68	1.35	12.710	8.59
3	RP	5146										5146	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	14666	14814									14740	2	104.2	0.71	73.6	0.50	1.00	12.710	6.35
10	RP	8409	8929									8669	2	367.7	4.24	260.0	3.00	6.00	12.710	38.12
11	RP	6337	6275									6306	2	44.0	0.70	31.1	0.49	0.99	12.710	6.27
12	RP	7951	8023									7987	2	51.0	0.64	36.1	0.45	0.90	12.710	5.74
13	RP	5298										5298	1							
14	RP	4309										4309	1							
15	RP	3014	3059									3036	2	32.0	1.05	22.6	0.74	1.49	12.710	9.46
D-Arg peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP																			
2	RP	795	789									792	2	3.6	0.46	2.6	0.33	0.65	12.710	4.13
3	RP	947										947	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	2257	2465									2361	2	147.4	6.24	104.2	4.41	8.83	12.710	56.10
10	RP	1254	1401									1328	2	104.4	7.86	73.8	5.56	11.12	12.710	70.66
11	RP	804	786									795	2	12.2	1.54	8.6	1.09	2.17	12.710	13.80
12	RP	967	983									975	2	11.6	1.19	8.2	0.84	1.68	12.710	10.70
13	RP	655										655	1							
14	RP	578										578	1							
15	RP	388	430									409	2	29.7	7.26	21.0	5.13	10.26	12.710	65.23

Table 4.12: Summary Statistics for L and D **Arginine** Concentration Data (pM)

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Arg Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP																			
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	2624	2756									2690	2	92.7	3.45	65.5	2.44	4.87	12.710	30.97
10	RP	2863	2892									2878	2	20.5	0.71	14.5	0.50	1.01	12.710	6.41
11	RP	2624	2655									2640	2	22.4	0.85	15.8	0.60	1.20	12.710	7.63
12	RP	4002	3982									3992	2	13.8	0.35	9.8	0.25	0.49	12.710	3.12
13	RP	3899										3899	1							
14	RP	4811										4811	1							
15	RP	3495	3562									3528	2	47.2	1.34	33.4	0.95	1.89	12.710	12.02
D-Arg Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP																			
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	404	459									431	2	38.7	8.97	27.4	6.35	12.69	12.710	80.66
10	RP	427	454									440	2	19.1	4.34	13.5	3.07	6.14	12.710	39.00
11	RP	333	333									333	2	0.0	0.01	0.0	0.01	0.02	12.710	0.10
12	RP	487	488									487	2	1.0	0.20	0.7	0.14	0.29	12.710	1.84
13	RP	482										482	1							
14	RP	645										645	1							
15	RP	450	501									475	2	35.8	7.54	25.3	5.33	10.67	12.710	67.78

Table 4.13: Summary Statistics for L and D **Arginine** D/L Ratio Value

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Arg		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP																			
2	RP	0.125	0.126									0.125	2	0.0006	0.50	0.0004	0.35	0.70	12.710	4.46
3	RP	0.184										0.184	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	0.154	0.166									0.160	2	0.0089	5.54	0.0063	3.92	7.83	12.710	49.76
10	RP	0.149	0.157									0.153	2	0.0056	3.63	0.0039	2.56	5.13	12.710	32.60
11	RP	0.127	0.125									0.126	2	0.0011	0.84	0.0007	0.59	1.18	12.710	7.53
12	RP	0.122	0.123									0.122	2	0.0007	0.55	0.0005	0.39	0.78	12.710	4.96
13	RP	0.124										0.124	1							
14	RP	0.134										0.134	1							
15	RP	0.129	0.141									0.135	2	0.0084	6.21	0.0059	4.39	8.78	12.710	55.79

Figure 4.11: Distribution of D/L Values submitted for **Arginine**

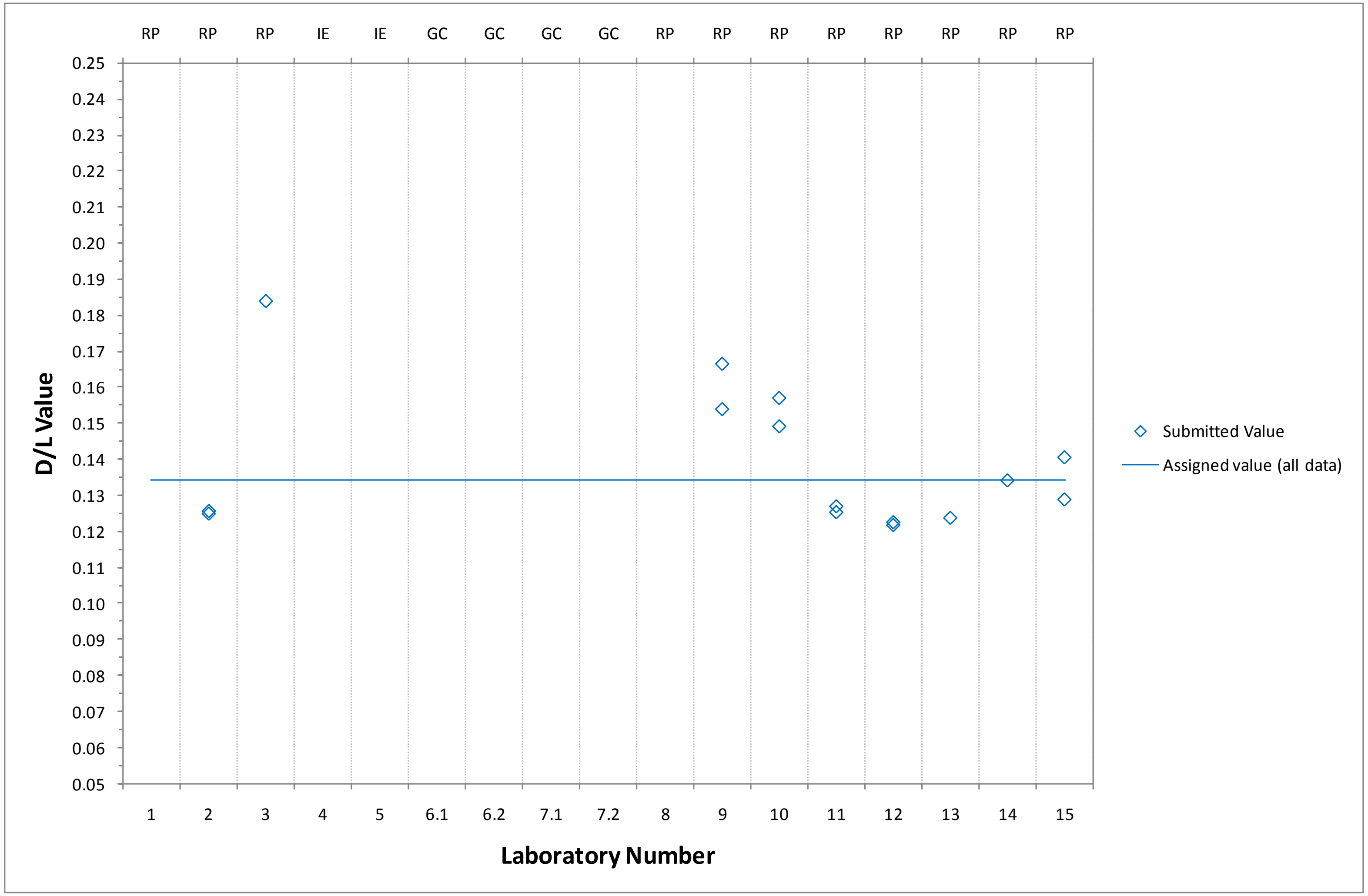


Figure 4.12: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Arginine** (value of n displayed).

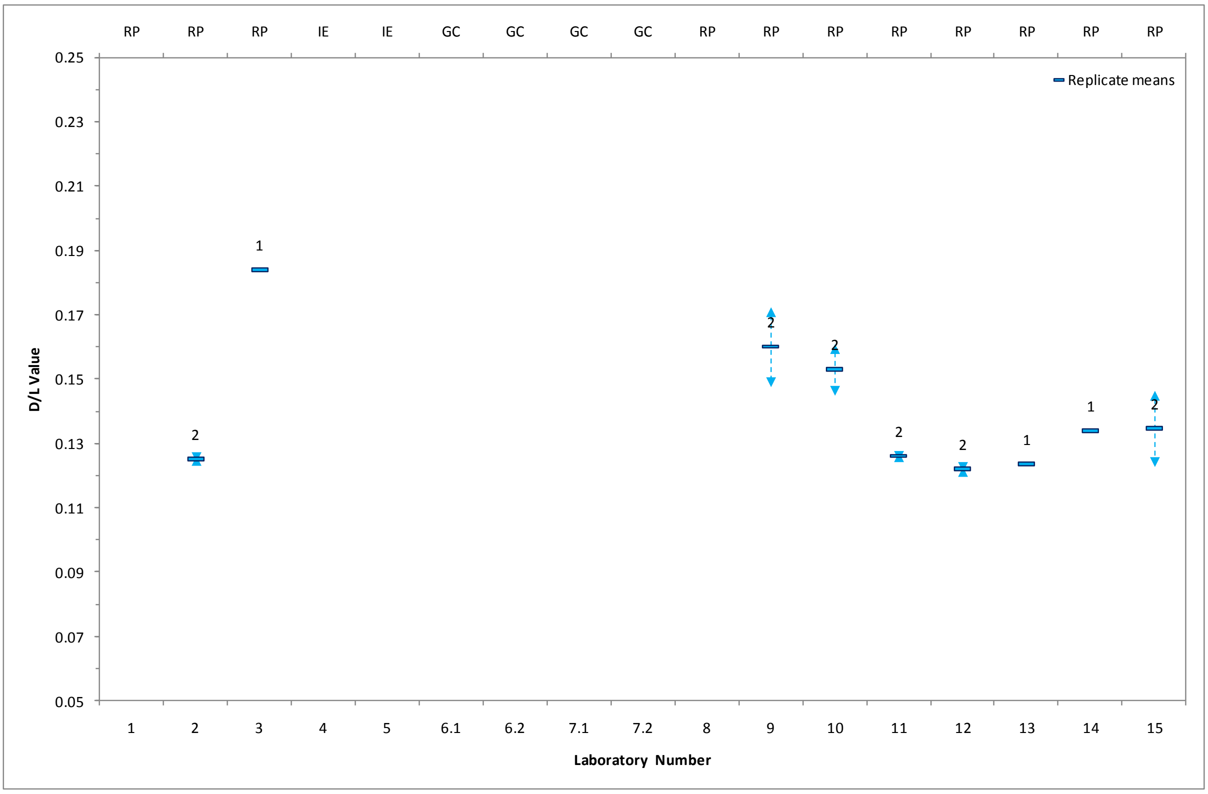


Figure 4.13: Experimental Expanded Uncertainty **(k=t$_{(0.05,df)}$)** of the Mean D/L value for **Arginine** (value of n displayed).

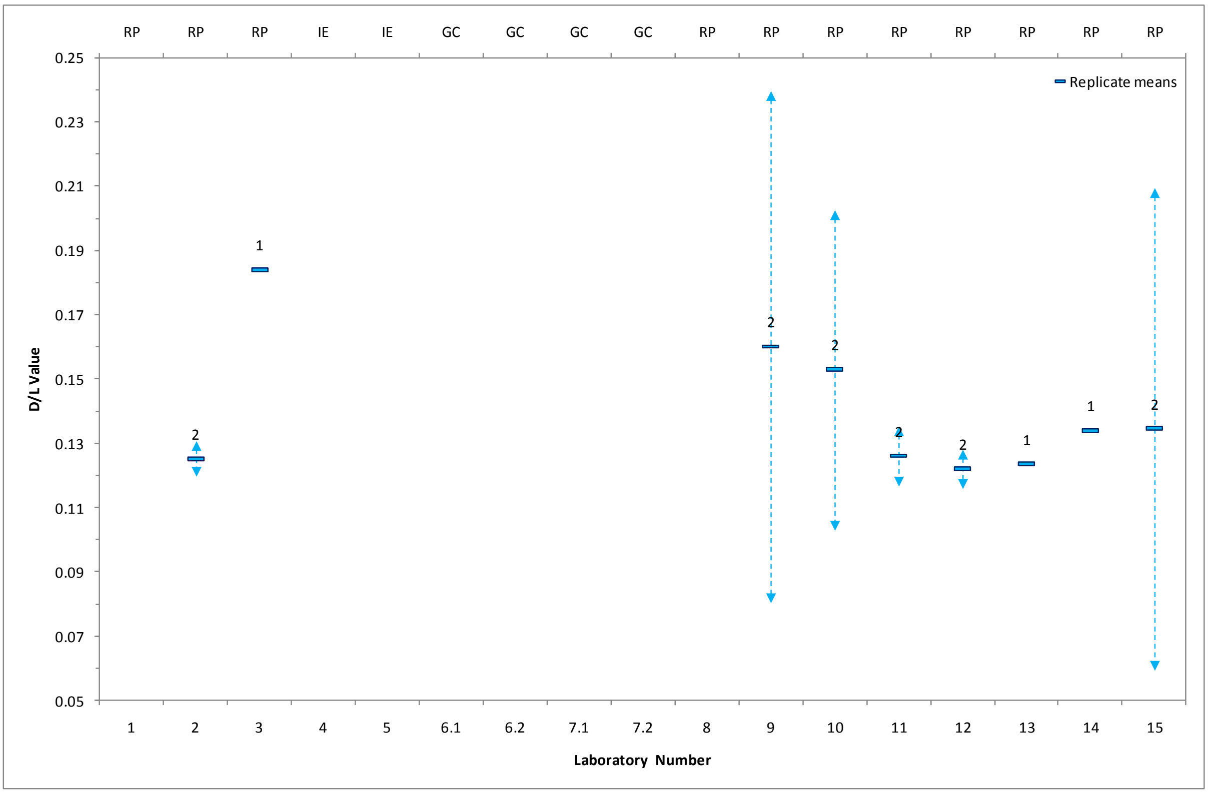


Table 4.14: Summary Statistics for L and D **Alanine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Ala peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	85745	87578	95663	102707	23575	25832	27120	28513	32191	34406	54333	10	33653.5	61.94	10642.2	19.59	39.17	2.262	44.31
2	RP	10966	10943									10954	2	15.7	0.14	11.1	0.10	0.20	12.710	1.29
3	RP	9103										9103	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	29305	29196									29250	2	77.1	0.26	54.5	0.19	0.37	12.710	2.37
9	RP	24173	24931									24552	2	536.1	2.18	379.0	1.54	3.09	12.710	19.62
10	RP	14138	15203									14671	2	753.3	5.13	532.7	3.63	7.26	12.710	46.15
11	RP	10066	9918									9992	2	104.8	1.05	74.1	0.74	1.48	12.710	9.43
12	RP	12382	12604									12493	2	156.7	1.25	110.8	0.89	1.77	12.710	11.27
13	RP	8675										8675	1							
14	RP	6691										6691	1							
15	RP	5078	5224									5151	2	103.1	2.00	72.9	1.41	2.83	12.710	17.98
D-Ala peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	8701	8786	8897	9568	2163	2331	2498	2633	2992	3267	5184	10	3296.6	63.60	1042.5	20.11	40.22	2.262	45.50
2	RP	923	925									924	2	1.4	0.15	1.0	0.11	0.21	12.710	1.34
3	RP	982										982	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	2633	2628									2631	2	3.5	0.13	2.5	0.09	0.19	12.710	1.21
9	RP	2706	2816									2761	2	77.7	2.81	54.9	1.99	3.98	12.710	25.29
10	RP	1660	1747									1704	2	61.6	3.62	43.6	2.56	5.12	12.710	32.52
11	RP	1168	1077									1123	2	64.5	5.75	45.6	4.06	8.13	12.710	51.65
12	RP	1519	1546									1532	2	18.8	1.23	13.3	0.87	1.73	12.710	11.02
13	RP	867										867	1							
14	RP	804										804	1							
15	RP	515	553									534	2	27.0	5.05	19.1	3.57	7.15	12.710	45.42

Table 4.15: Summary Statistics for L and D **Alanine** Concentration Data (pM)

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Ala Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	4503	4550	4379	4459	4623	4743	4659	4656	4603	4660	4584	10	110.1	2.40	34.8	0.76	1.52	2.262	1.72
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	4812	4774									4793	2	27.0	0.56	19.1	0.40	0.80	12.710	5.06
9	RP	4083	4378									4231	2	208.2	4.92	147.2	3.48	6.96	12.710	44.23
10	RP	4544	4649									4596	2	73.8	1.61	52.2	1.14	2.27	12.710	14.44
11	RP	3935	3963									3949	2	19.7	0.50	13.9	0.35	0.70	12.710	4.47
12	RP	5883	5906									5894	2	15.9	0.27	11.2	0.19	0.38	12.710	2.42
13	RP	6027										6027	1							
14	RP	7051										7051	1							
15	RP	5560	5742									5651	2	129.2	2.29	91.3	1.62	3.23	12.710	20.54
D-Ala Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	457	456	407	415	424	428	429	430	428	443	432	10	16.1	3.72	5.1	1.18	2.35	2.262	2.66
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	432	430									431	2	1.9	0.43	1.3	0.31	0.61	12.710	3.89
9	RP	387	419									403	2	22.4	5.55	15.8	3.93	7.85	12.710	49.90
10	RP	452	453									453	2	0.4	0.09	0.3	0.06	0.13	12.710	0.80
11	RP	387	365									376	2	15.8	4.20	11.2	2.97	5.94	12.710	37.77
12	RP	612	614									613	2	1.5	0.24	1.0	0.17	0.34	12.710	2.17
13	RP	510										510	1							
14	RP	718										718	1							
15	RP	478	515									497	2	26.5	5.34	18.7	3.77	7.55	12.710	47.98

Table 4.16: Summary Statistics for L and D **Alanine** D/L Ratio Value

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Ala		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	0.101	0.100	0.093	0.093	0.092	0.090	0.092	0.092	0.093	0.095	0.094	10	0.0037	3.95	0.0012	1.25	2.50	2.262	2.82
2	RP	0.084	0.085									0.084	2	0.0002	0.29	0.0002	0.21	0.41	12.710	2.63
3	RP	0.108										0.108	1							
4	IE																			
5	IE																			
6.1[1]	GC_A	0.077										0.077	7	0.0040	5.19	0.0015	1.96	3.93	2.447	4.80
6.2[1]	GC_H	0.072										0.072	5	0.0010	1.39	0.0004	0.62	1.24	2.777	1.72
7.1[1]	GC_A	0.077										0.077	1							
7.2	GC																			
8	RP	0.090	0.090									0.090	2	0.0000	0.00	0.0000	0.00	0.00	12.710	0.00
9	RP	0.095	0.096									0.095	2	0.0006	0.63	0.0004	0.45	0.89	12.710	5.67
10	RP	0.100	0.097									0.098	2	0.0015	1.52	0.0011	1.07	2.15	12.710	13.64
11	RP	0.098	0.092									0.095	2	0.0045	4.70	0.0032	3.32	6.65	12.710	42.24
12	RP	0.104	0.104									0.104	2	0.0000	0.03	0.0000	0.02	0.04	12.710	0.25
13	RP	0.085										0.085	1							
14	RP	0.102										0.102	1							
15	RP	0.086	0.090									0.088	2	0.0027	3.05	0.0019	2.16	4.32	12.710	27.45

[1]= submitted as the mean and standard deviation of n results.

GC_A = derived using peak area

GC_H = derived using peak height

Figure 4.14: Distribution of D/L Values submitted for **Alanine**

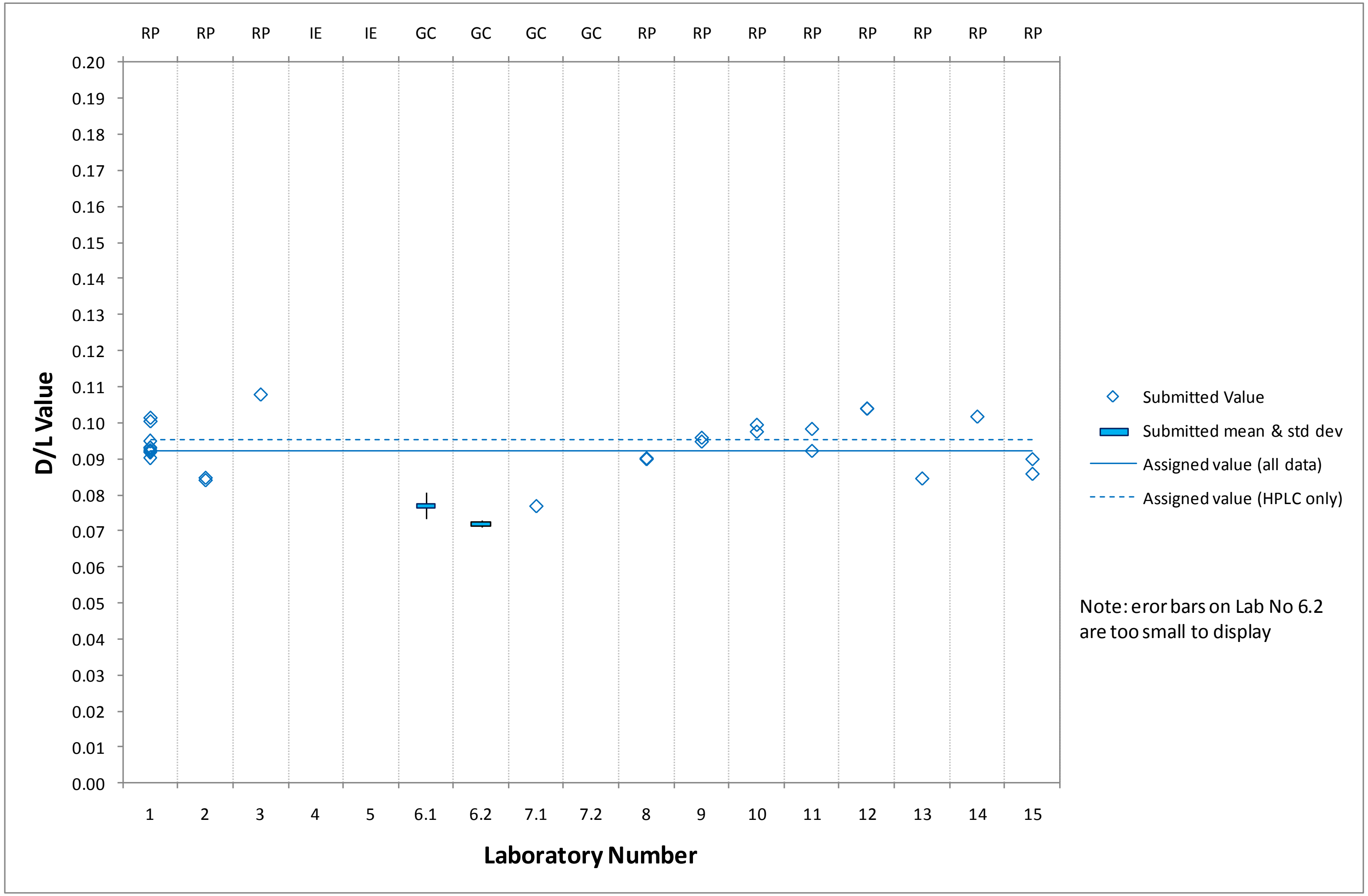


Figure 4.15: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Alanine** (value of n displayed).

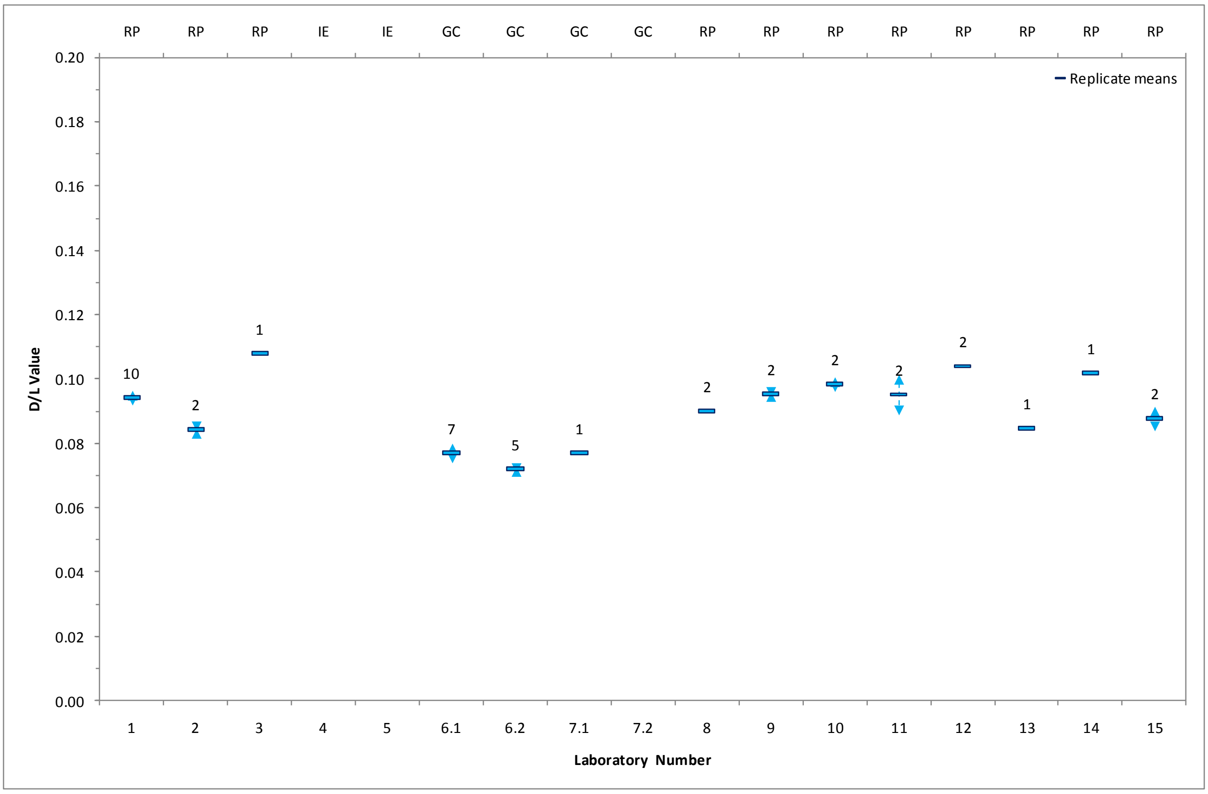


Figure 4.16: Experimental Expanded Uncertainty **($k=t_{(0.05,df)}$)** of the Mean D/L value for **Alanine** (value of n displayed).

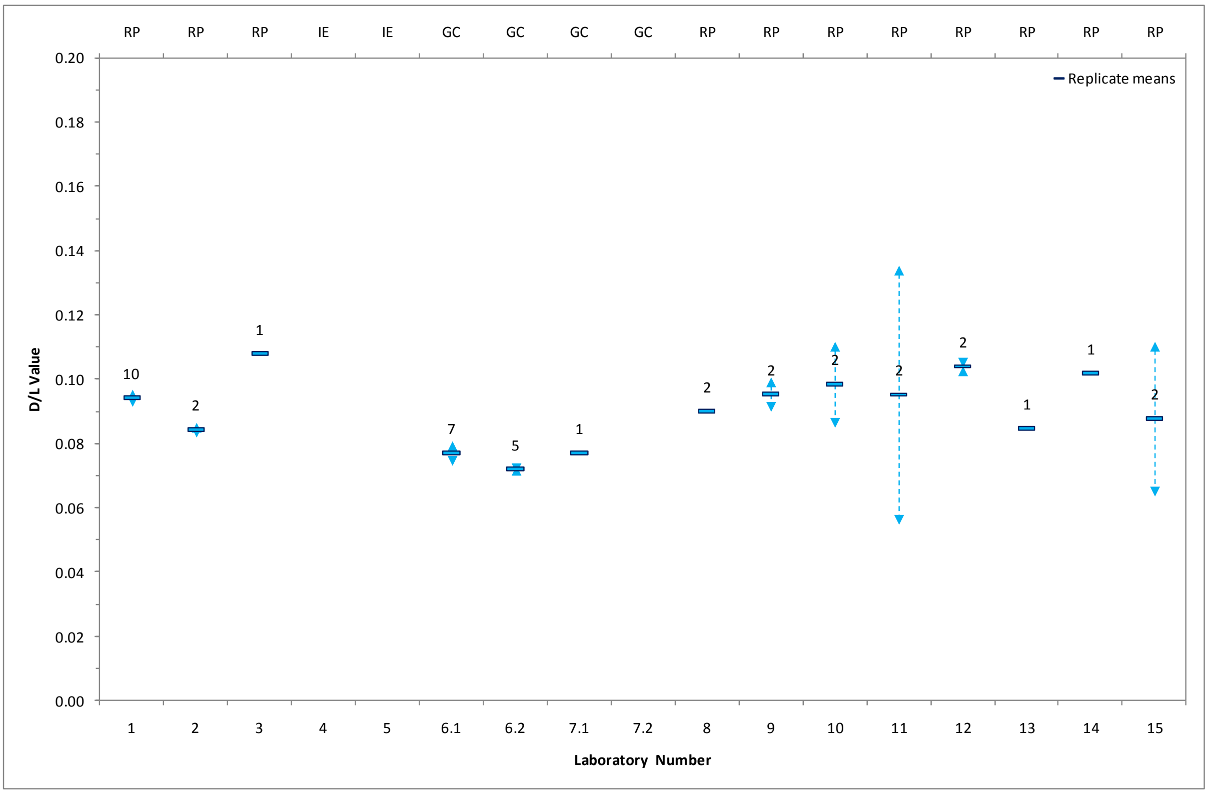


Table 4.17: Summary Statistics for L and D **Valine** Peak Area / Height Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Val peak area		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	46869	47640	48552	54051	12253	13452	14172	15087	16694	17888	28666	10	17907.2	62.47	5662.7	19.75	39.51	2.262	44.69
2	RP	5442	5411									5427	2	22.2	0.41	15.7	0.29	0.58	12.710	3.67
3	RP	4505										4505	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	15441	15360									15400	2	56.9	0.37	40.2	0.26	0.52	12.710	3.32
9	RP	14669	15173									14921	2	356.7	2.39	252.2	1.69	3.38	12.710	21.49
10	RP	8285	8986									8635	2	496.0	5.74	350.7	4.06	8.12	12.710	51.62
11	RP	5927	5842									5885	2	60.6	1.03	42.9	0.73	1.46	12.710	9.26
12	RP	7449	7563									7506	2	80.2	1.07	56.7	0.76	1.51	12.710	9.61
13	RP	5139										5139	1							
14	RP	3896										3896	1							
15	RP	2941	3030									2985	2	62.6	2.10	44.3	1.48	2.96	12.710	18.84
D-Val peak area		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	1051	1023	1303	1454	318	355	390	435	471	554	735	10	428.4	58.26	135.5	18.42	36.85	2.262	41.68
2	RP	163	159									161	2	2.8	1.74	2.0	1.23	2.46	12.710	15.64
3	RP	147										147	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	490	481									486	2	6.7	1.38	4.7	0.97	1.95	12.710	12.38
9	RP	500	552									526	2	36.3	6.91	25.7	4.88	9.77	12.710	62.09
10	RP	304	321									312	2	11.8	3.79	8.4	2.68	5.36	12.710	34.07
11	RP	197	187									192	2	6.9	3.58	4.9	2.53	5.07	12.710	32.21
12	RP	222	228									225	2	4.7	2.09	3.3	1.48	2.96	12.710	18.79
13	RP	133										133	1							
14	RP	156										156	1							
15	RP	91	99									95	2	5.9	6.24	4.2	4.42	8.83	12.710	56.12
D+L Val peak height		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
004	IE	8.645	12.413	12.668								11.242	3	2.2527	20.04	1.3006	11.57	23.14	4.303	49.78
005	IE	14.193	14.204									14.199	2	0.0078	0.05	0.0055	0.04	0.08	12.710	0.49

Table 4.18: Summary Statistics for L and D **Valine** Concentration Data (pM)

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Val Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	2461	2475	2222	2347	2403	2470	2435	2464	2387	2423	2409	10	77.4	3.21	24.5	1.02	2.03	2.262	2.30
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	2536	2512									2524	2	16.9	0.67	11.9	0.47	0.95	12.710	6.01
9	RP	2284	2456									2370	2	121.6	5.13	86.0	3.63	7.25	12.710	46.09
10	RP	2455	2533									2494	2	55.3	2.22	39.1	1.57	3.13	12.710	19.92
11	RP	2136	2151									2144	2	11.1	0.52	7.8	0.36	0.73	12.710	4.64
12	RP	3263	3266									3264	2	2.7	0.08	1.9	0.06	0.12	12.710	0.75
13	RP	3292										3292	1							
14	RP	3784										3784	1							
15	RP	2968	3070									3019	2	71.9	2.38	50.8	1.68	3.37	12.710	21.40
D-Val Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	55	53	60	63	62	65	67	71	67	75	64	10	6.8	10.56	2.1	3.34	6.68	2.262	7.56
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	81	79									80	2	1.3	1.68	0.9	1.19	2.37	12.710	15.07
9	RP	70	80									75	2	7.3	9.64	5.1	6.82	13.63	12.710	86.63
10	RP	81	81									81	2	0.2	0.26	0.2	0.18	0.37	12.710	2.35
11	RP	64	62									63	2	1.3	2.04	0.9	1.44	2.88	12.710	18.31
12	RP	87	89									88	2	1.0	1.11	0.7	0.78	1.56	12.710	9.93
13	RP	77										77	1							
14	RP	136										136	1							
15	RP	82	90									86	2	5.6	6.53	4.0	4.62	9.23	12.710	58.68

Table 4.19: Summary Statistics for L and D **Valine** D/L Ratio Value

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Valine		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% (k=t_{crit})**
1	RP	0.022	0.021	0.027	0.027	0.026	0.026	0.028	0.029	0.028	0.031	0.027	10	0.0028	10.64	0.0009	3.37	6.73	2.262	7.61
2	RP	0.030	0.029									0.030	2	0.0004	1.33	0.0003	0.94	1.88	12.710	11.97
3	RP	0.033										0.033	1							
4	IE																			
5	IE																			
6.1[1]	GC_A	0.019										0.019	6	0.0030	15.79	0.0012	6.45	12.89	2.571	16.57
6.2[1]	GC_H	0.019										0.019	3	0.0010	5.26	0.0006	3.04	6.08	4.303	13.07
7.1[1]	GC_A	0.030										0.030	1							
7.2[1]	GC_H	0.022										0.022	1							
8	RP	0.032	0.031									0.032	2	0.0007	2.24	0.0005	1.59	3.17	12.710	20.17
9	RP	0.031	0.033									0.032	2	0.0014	4.52	0.0010	3.20	6.39	12.710	40.64
10	RP	0.033	0.032									0.033	2	0.0006	1.95	0.0005	1.38	2.76	12.710	17.57
11	RP	0.030	0.029									0.029	2	0.0007	2.55	0.0005	1.81	3.61	12.710	22.95
12	RP	0.027	0.027									0.027	2	0.0003	1.02	0.0002	0.72	1.44	12.710	9.18
13	RP	0.023										0.023	1							
14	RP	0.036										0.036	1							
15	RP	0.028	0.029									0.029	2	0.0012	4.15	0.0008	2.94	5.87	12.710	37.31

[1]= submitted as the mean and standard deviation of n results.

GC_A = derived using peak area

GC_H = derived using peak height

Figure 4.17: Distribution of D/L Values submitted for **Valine**

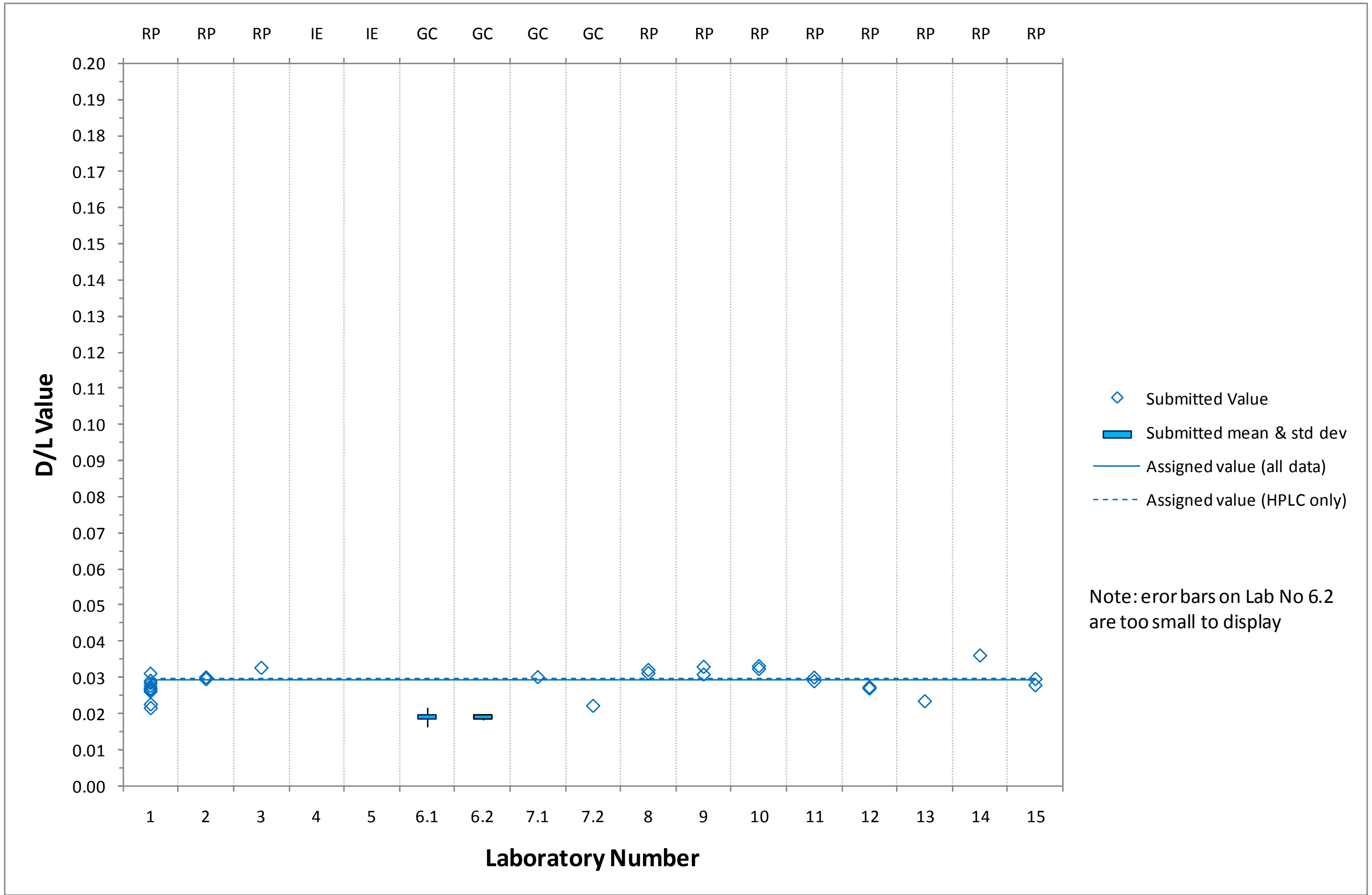


Figure 4.18: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Valine** (value of n displayed).

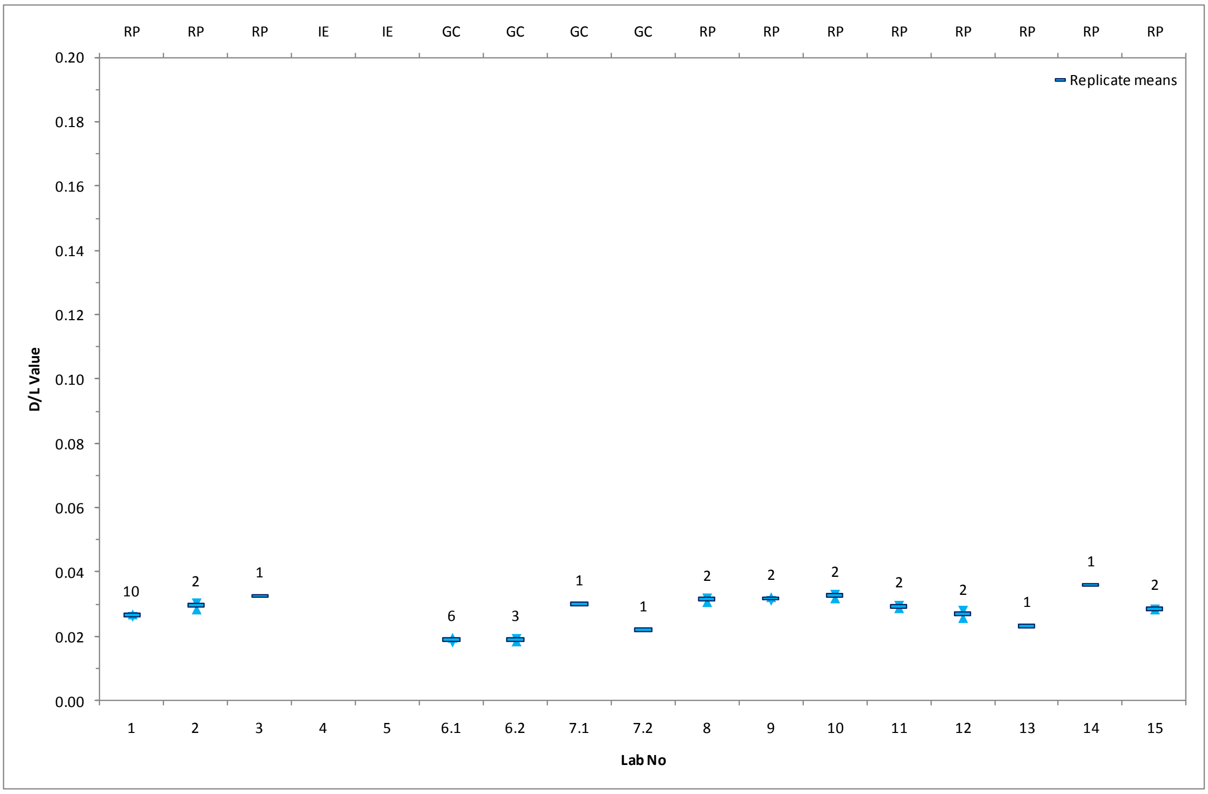


Figure 4.19: Experimental Expanded Uncertainty **($k=t_{(0.05,df)}$)** of the Mean D/L value for **Valine** (value of n displayed).

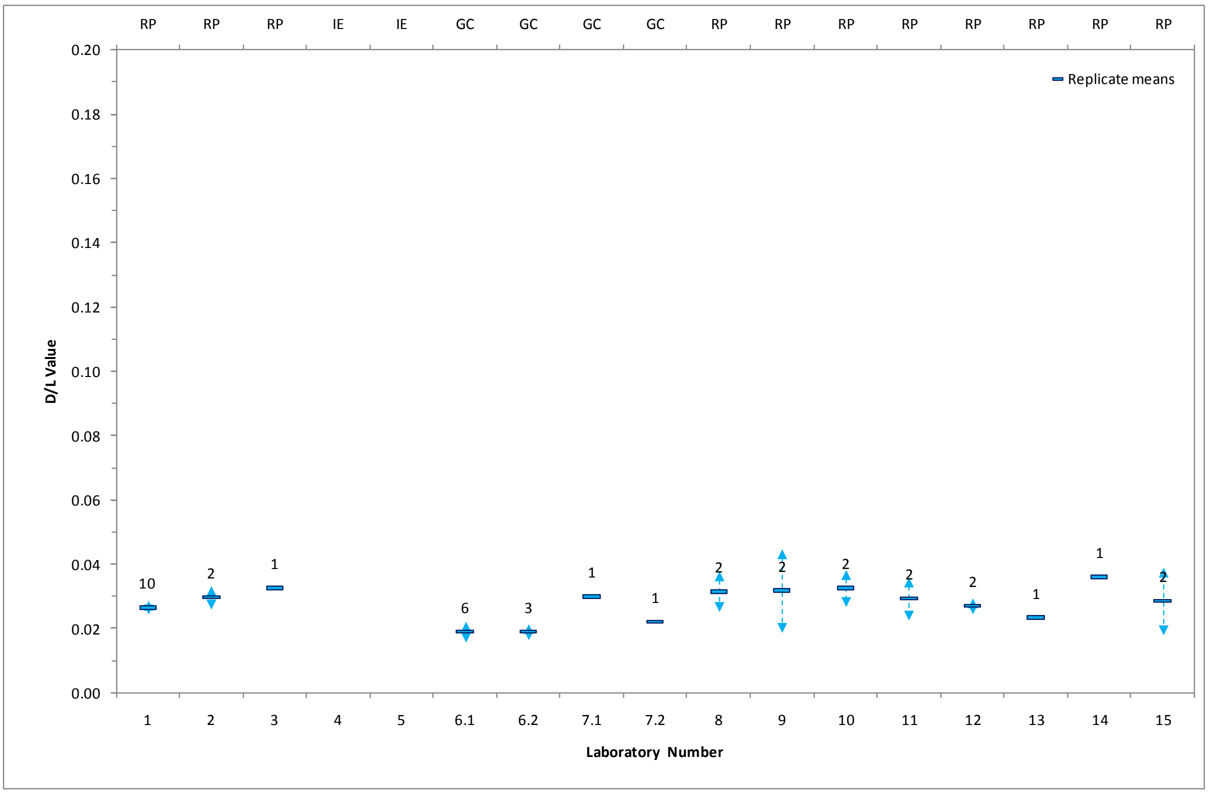


Table 4.20: Summary Statistics for L and D **Phenylalanine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Phe peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	44275	45100	48703	52473	11909	12965	13645	14414	16078	17276	27684	10	17373.5	62.76	5494.0	19.85	39.69	2.262	44.89
2	RP	5587	5555									5571	2	22.2	0.40	15.7	0.28	0.56	12.710	3.58
3	RP	4745										4745	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	15000	13905									14453	2	773.7	5.35	547.1	3.79	7.57	12.710	48.11
9	RP	12443	12717									12580	2	193.7	1.54	137.0	1.09	2.18	12.710	13.84
10	RP	7171	7593									7382	2	298.1	4.04	210.8	2.86	5.71	12.710	36.29
11	RP	5102	5025									5064	2	54.4	1.08	38.5	0.76	1.52	12.710	9.66
12	RP	6493	6619									6556	2	88.9	1.36	62.8	0.96	1.92	12.710	12.18
13	RP	4563										4563	1							
14	RP	3400										3400	1							
15	RP	2578	2655									2616	2	54.4	2.08	38.5	1.47	2.94	12.710	18.68
D-Phe peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	3413	3574	3597	3834	858	956	1007	1074	1073	1293	2068	10	1330.8	64.35	420.8	20.35	40.70	2.262	46.04
2	RP	396	392									394	2	2.7	0.70	1.9	0.49	0.98	12.710	6.25
3	RP	380										380	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	1043	1041									1042	2	1.5	0.15	1.1	0.10	0.21	12.710	1.33
9	RP	1017	1041									1029	2	17.3	1.68	12.2	1.19	2.38	12.710	15.13
10	RP	598	626									612	2	19.9	3.25	14.1	2.30	4.60	12.710	29.24
11	RP	408	397									403	2	7.6	1.88	5.3	1.33	2.66	12.710	16.88
12	RP	528	536									532	2	5.7	1.06	4.0	0.75	1.50	12.710	9.56
13	RP	358										358	1							
14	RP	266										266	1							
15	RP	200	214									207	2	10.0	4.85	7.1	3.43	6.86	12.710	43.61

Table 4.21: Summary Statistics for L and D **Phenylalanine** Concentration Data (pM)

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Phe Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	2325	2343	2229	2278	2335	2381	2344	2354	2299	2340	2323	10	43.4	1.87	13.7	0.59	1.18	2.262	1.34
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	2463	2274									2369	2	133.9	5.65	94.7	4.00	7.99	12.710	50.80
9	RP	2115	2247									2181	2	93.3	4.28	66.0	3.03	6.05	12.710	38.46
10	RP	2319	2336									2328	2	11.9	0.51	8.4	0.36	0.72	12.710	4.58
11	RP	2007	2020									2013	2	9.5	0.47	6.7	0.33	0.67	12.710	4.24
12	RP	3104	3120									3112	2	11.5	0.37	8.1	0.26	0.52	12.710	3.33
13	RP	3190										3190	1							
14	RP	3605										3605	1							
15	RP	2840	2936									2888	2	68.3	2.36	48.3	1.67	3.34	12.710	21.24
D-Phe Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	179	186	165	166	168	176	173	175	153	175	172	10	8.9	5.20	2.8	1.65	3.29	2.262	3.72
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	171	170									171	2	0.8	0.45	0.5	0.32	0.63	12.710	4.02
9	RP	173	184									178	2	7.9	4.42	5.6	3.13	6.25	12.710	39.74
10	RP	193	193									193	2	0.5	0.28	0.4	0.20	0.39	12.710	2.48
11	RP	160	160									160	2	0.5	0.33	0.4	0.23	0.47	12.710	2.98
12	RP	252	253									253	2	0.2	0.08	0.1	0.06	0.11	12.710	0.71
13	RP	250										250	1							
14	RP	282										282	1							
15	RP	220	237									228	2	11.7	5.14	8.3	3.63	7.26	12.710	46.16

Table 4.22: Summary Statistics for L and D **Phenylalanine** D/L Ratio Value

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Phe		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	0.077	0.079	0.074	0.073	0.072	0.074	0.074	0.074	0.067	0.075	0.074	10	0.0033	4.41	0.0010	1.39	2.79	2.262	3.15
2	RP	0.071	0.071									0.071	2	0.0002	0.30	0.0001	0.21	0.42	12.710	2.67
3	RP	0.080										0.080	1							
4	IE																			
5	IE																			
6.1[1]	GC_A	0.067										0.067	7	0.0080	11.94	0.0030	4.51	9.03	2.447	11.04
6.2[1]	GC_H	0.090										0.090	1							
7.1[1]	GC_A	0.064										0.064	1							
7.2[1]	GC_H	0.067										0.067	1							
8	RP	0.075	0.088									0.082	2	0.0092	11.28	0.0065	7.98	15.95	12.710	101.37
9	RP	0.082	0.082									0.082	2	0.0001	0.14	0.0001	0.10	0.20	12.710	1.28
10	RP	0.083	0.083									0.083	2	0.0007	0.79	0.0005	0.56	1.11	12.710	7.06
11	RP	0.080	0.079									0.079	2	0.0006	0.80	0.0005	0.57	1.14	12.710	7.22
12	RP	0.081	0.081									0.081	2	0.0002	0.29	0.0002	0.21	0.41	12.710	2.62
13	RP	0.078										0.078	1							
14	RP	0.078										0.078	1							
15	RP	0.077	0.081									0.079	2	0.0022	2.77	0.0016	1.96	3.92	12.710	24.94

[1]= submitted as the mean and standard deviation of n results.

GC_A = derived using peak area

GC_H = derived using peak height

Figure 4.20: Distribution of D/L Values submitted for **Phenylalanine**

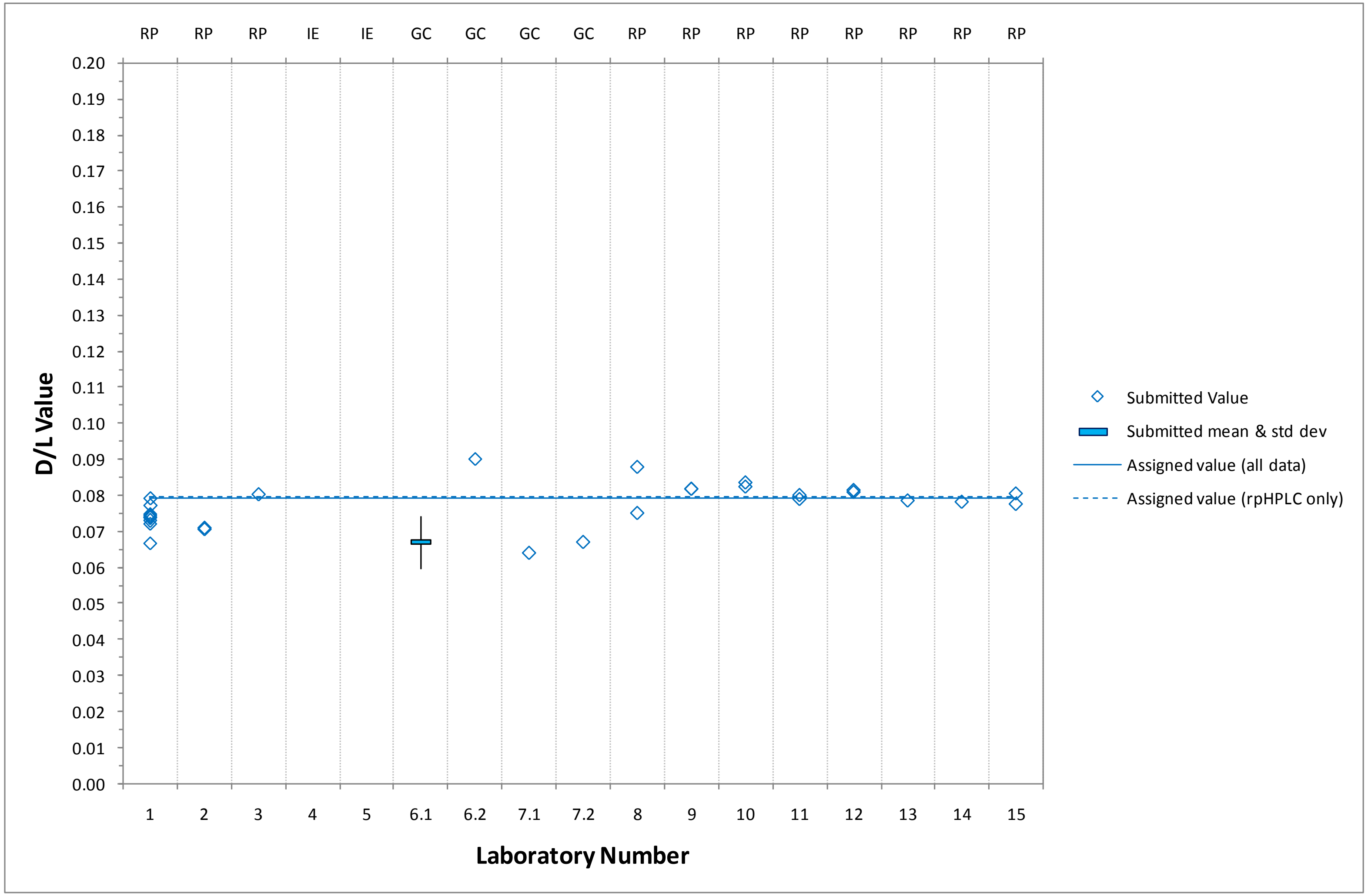


Figure 4.21: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Phenylalanine** (value of n displayed).

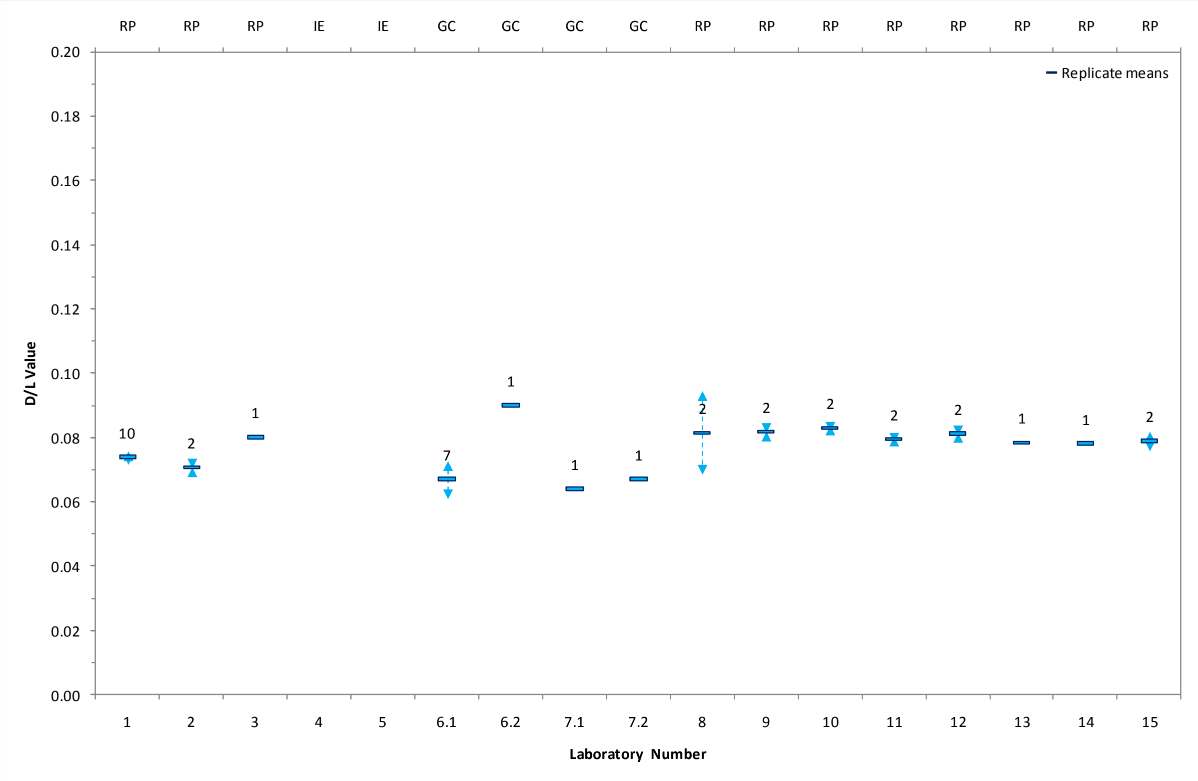


Figure 4.22: Experimental Expanded Uncertainty **($k=t_{(0.05,df)}$)** of the Mean D/L value for **Phenylalanine** (value of n displayed).

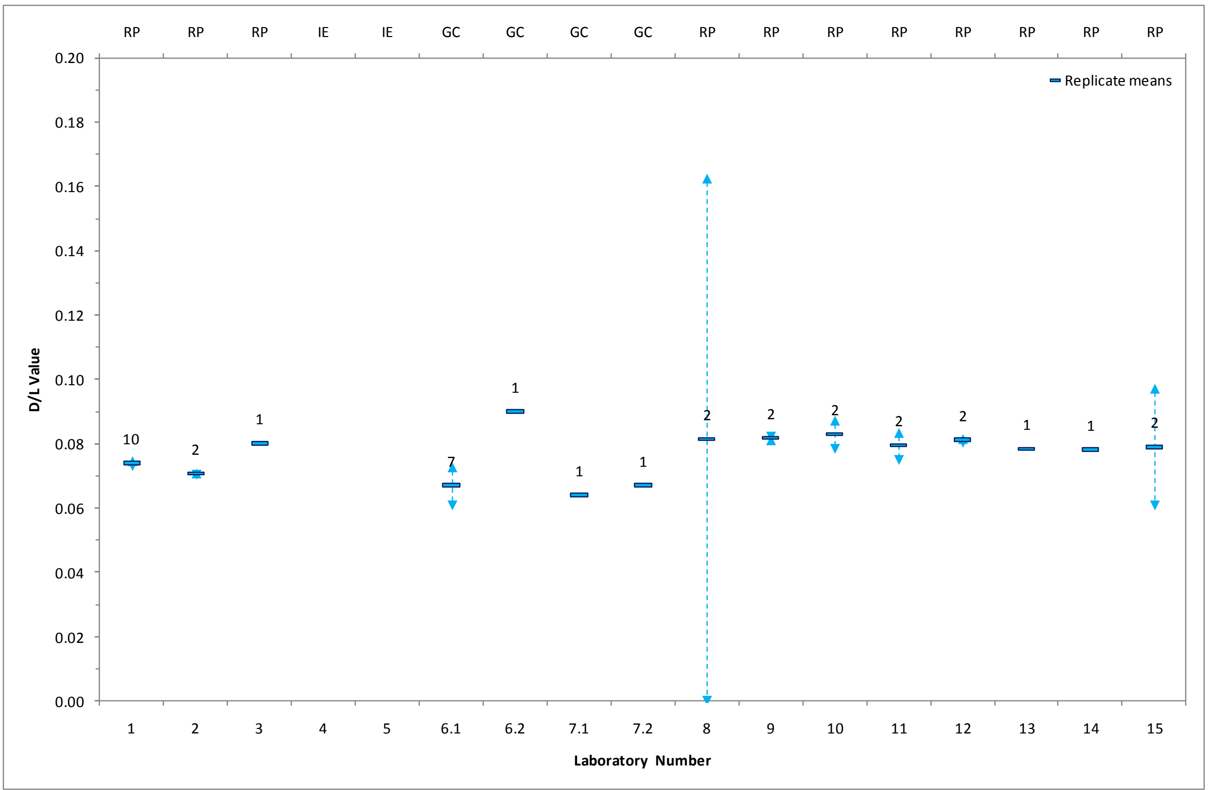


Table 4.23: Summary Statistics for **D-Alloisoleucine/L-Isoleucine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Ile peak area*		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% (k=t_{crit})**
1	RP	53647	54562	56495	62468	14114	15505	16385	17363	19374	20725	33064	10	20632.2	62.40	6524.5	19.73	39.47	2.262	44.64
2	RP	6074	6044									6059	2	21.2	0.35	15.0	0.25	0.50	12.710	3.15
3	RP	4996										4996	1							
4	IE*	8.429	3.870	3.878								5.392	3	2.63	48.77	1.5	28.16	56.31	4.303	121.15
5	IE*	3.949	3.928									3.939	2	0.015	0.38	0.011	0.27	0.53	12.710	3.39
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	17678	17496									17587	2	128.6	0.73	90.9	0.52	1.03	12.710	6.57
9	RP	16595	17062									16828	2	330.3	1.96	233.6	1.39	2.78	12.710	17.64
10	RP	9473	10122									9798	2	458.6	4.68	324.3	3.31	6.62	12.710	42.07
11	RP	6757	6657									6707	2	70.9	1.06	50.2	0.75	1.50	12.710	9.50
12	RP	8620	8773									8696	2	108.2	1.24	76.5	0.88	1.76	12.710	11.18
13	RP	6045										6045	1							
14	RP	4474										4474	1							
15	RP	3355	3451									3403	2	68.1	2.00	48.1	1.41	2.83	12.710	17.98
D-Aile peak area*		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% (k=t_{crit})**
1	RP	1500	1516	1443	1605	332	392	429	468	502	515	870	10	559.6	64.31	177.0	20.34	40.67	2.262	46.00
2	RP	240	235									238	2	3.3	1.39	2.3	0.99	1.97	12.710	12.53
3	RP	317										317	1							
4	IE*	0.287	0.117	0.117								0.174	3	0.1	56.52	0.1	32.63	65.26	4.303	140.40
5	IE*	0.122	0.122									0.122	2	0.0	0.00	0.0	0.00	0.00	12.710	0.00
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	461	460									461	2	1.2	0.26	0.8	0.18	0.37	12.710	2.33
9	RP	684	807									745	2	86.6	11.62	61.3	8.22	16.43	12.710	104.43
10	RP	425	486									455	2	42.9	9.42	30.3	6.66	13.32	12.710	84.63
11	RP	274	266									270	2	5.2	1.94	3.7	1.37	2.74	12.710	17.42
12	RP	332	344									338	2	8.5	2.51	6.0	1.77	3.55	12.710	22.54
13	RP	213										213	1							
14	RP	199										199	1							
15	RP	126	138									132	2	8.2	6.23	5.8	4.40	8.81	12.710	55.98

* = peak height data

Table 4.24: Summary Statistics for **D-Alloisoleucine/L-Isoleucine** Concentration Data (pM)

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Ile Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% (k=t_{crit})**
1	RP	2817	2835	2586	2712	2767	2847	2815	2835	2770	2807	2779	10	79.1	2.85	25.0	0.90	1.80	2.262	2.04
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	2903	2861									2882	2	29.7	1.03	21.0	0.73	1.46	12.710	9.26
9	RP	2682	2866									2774	2	130.4	4.70	92.2	3.32	6.65	12.710	42.25
10	RP	2913	2961									2937	2	33.8	1.15	23.9	0.81	1.63	12.710	10.36
11	RP	2527	2544									2535	2	12.4	0.49	8.8	0.35	0.69	12.710	4.40
12	RP	3918	3932									3925	2	10.2	0.26	7.2	0.18	0.37	12.710	2.33
13	RP	4018										4018	1							
14	RP	4510										4510	1							
15	RP	3513	3629									3571	2	81.6	2.29	57.7	1.62	3.23	12.710	20.54
D-Aile Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% (k=t_{crit})**
1	RP	79	79	66	70	65	72	74	76	72	70	72	10	4.8	6.66	1.5	2.11	4.21	2.262	4.77
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	76	75									75	2	0.4	0.56	0.3	0.39	0.79	12.710	5.02
9	RP	111	136									123	2	17.6	14.34	12.5	10.14	20.28	12.710	128.85
10	RP	131	142									136	2	8.0	5.90	5.7	4.17	8.34	12.710	52.99
11	RP	102	102									102	2	0.4	0.39	0.3	0.28	0.55	12.710	3.52
12	RP	151	154									153	2	2.3	1.52	1.6	1.08	2.15	12.710	13.68
13	RP	142										142	1							
14	RP	201										201	1							
15	RP	132	145									138	2	9.0	6.51	6.4	4.61	9.21	12.710	58.53

Table 4.25: Summary Statistics for **D-Alloisoleucine/L-Isoleucine** D/L Ratio Value

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Aile/Ile		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	0.028	0.028	0.026	0.026	0.023	0.025	0.026	0.027	0.026	0.025	0.026	10	0.0013	5.19	0.0004	1.64	3.28	2.262	3.71
2	RP	0.040	0.039									0.039	2	0.0004	1.04	0.0003	0.74	1.48	12.710	9.38
3	RP	0.063										0.063	1							
4	IE	0.034	0.030	0.030								0.031	3	0.0023	7.37	0.0013	4.26	8.51	4.303	18.31
5	IE	0.031	0.031									0.031	2	0.0000	0.00	0.0000	0.00	0.00	12.710	0.00
6.1[1]	GC_A	0.033										0.033	7	0.0020	6.06	0.0008	2.29	4.58	2.447	5.61
6.2[1]	GC_H	0.036										0.036	6	0.0030	8.33	0.0012	3.40	6.80	2.571	8.75
7.1[1]	GC_A	0.044										0.044	1							
7.2[1]	GC_H	0.041										0.041	1							
8	RP	0.026	0.030									0.028	2	0.0028	10.10	0.0020	7.14	14.29	12.710	90.79
9	RP	0.041	0.047									0.044	2	0.0043	9.67	0.0030	6.84	13.67	12.710	86.89
10	RP	0.045	0.048									0.046	2	0.0022	4.75	0.0016	3.36	6.71	12.710	42.65
11	RP	0.041	0.040									0.040	2	0.0004	0.88	0.0003	0.62	1.25	12.710	7.91
12	RP	0.039	0.039									0.039	2	0.0005	1.26	0.0003	0.89	1.79	12.710	11.36
13	RP	0.035										0.035	1							
14	RP	0.045										0.045	1							
15	RP	0.038	0.040									0.039	2	0.0016	4.23	0.0012	2.99	5.98	12.710	38.02

[1]= submitted as the mean and standard deviation of n results.

GC_A = derived using peak area

GC_H = derived using peak height

Figure 4.23: Distribution of D/L Values submitted for **D-Alloisoleucine/L-Isoleucine**

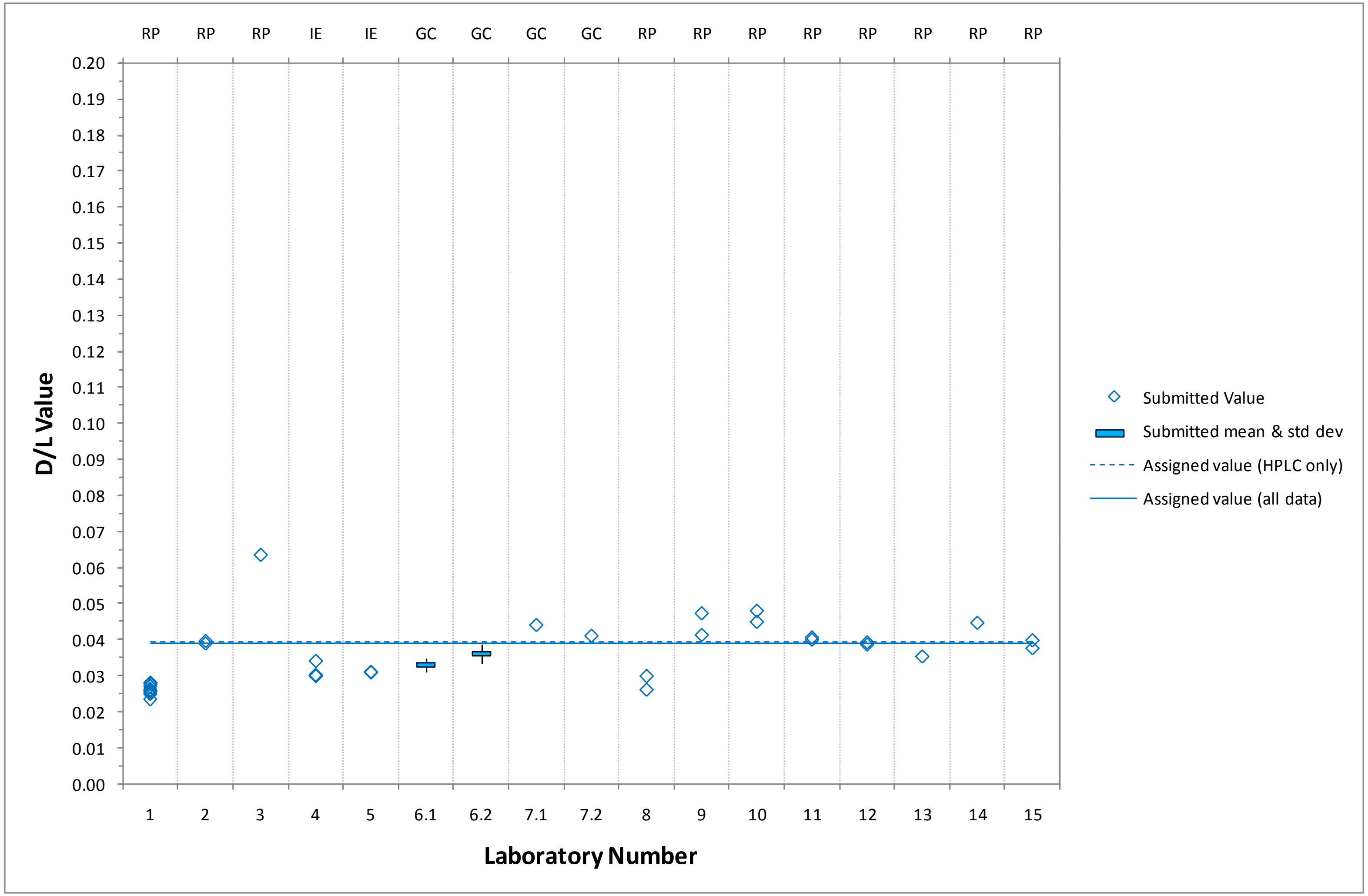


Figure 4.24: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **D-Alloisoleucine/L-Isoleucine** (value of n displayed).

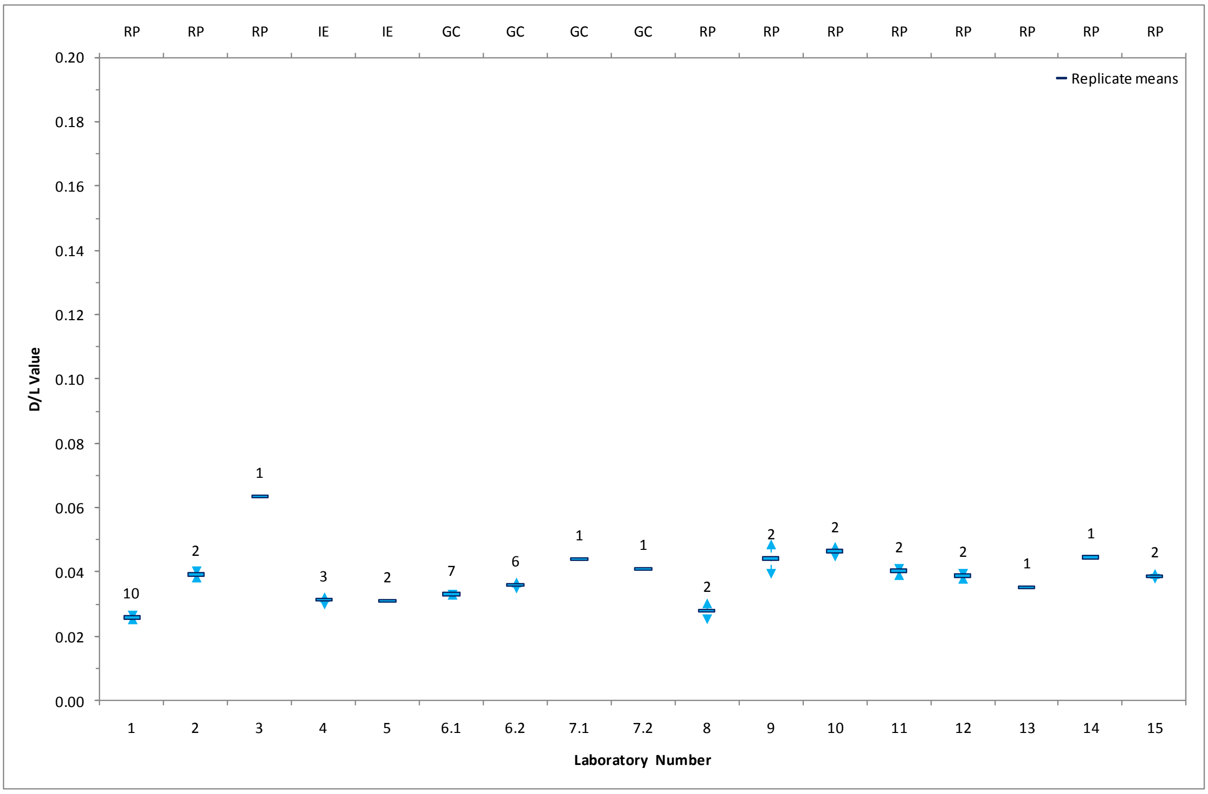


Figure 4.25: Experimental Expanded Uncertainty **(k=$t_{(0.05,df)}$)** of the Mean D/L value for **D-Alloisoleucine/L-Isoleucine** (value of n displayed).

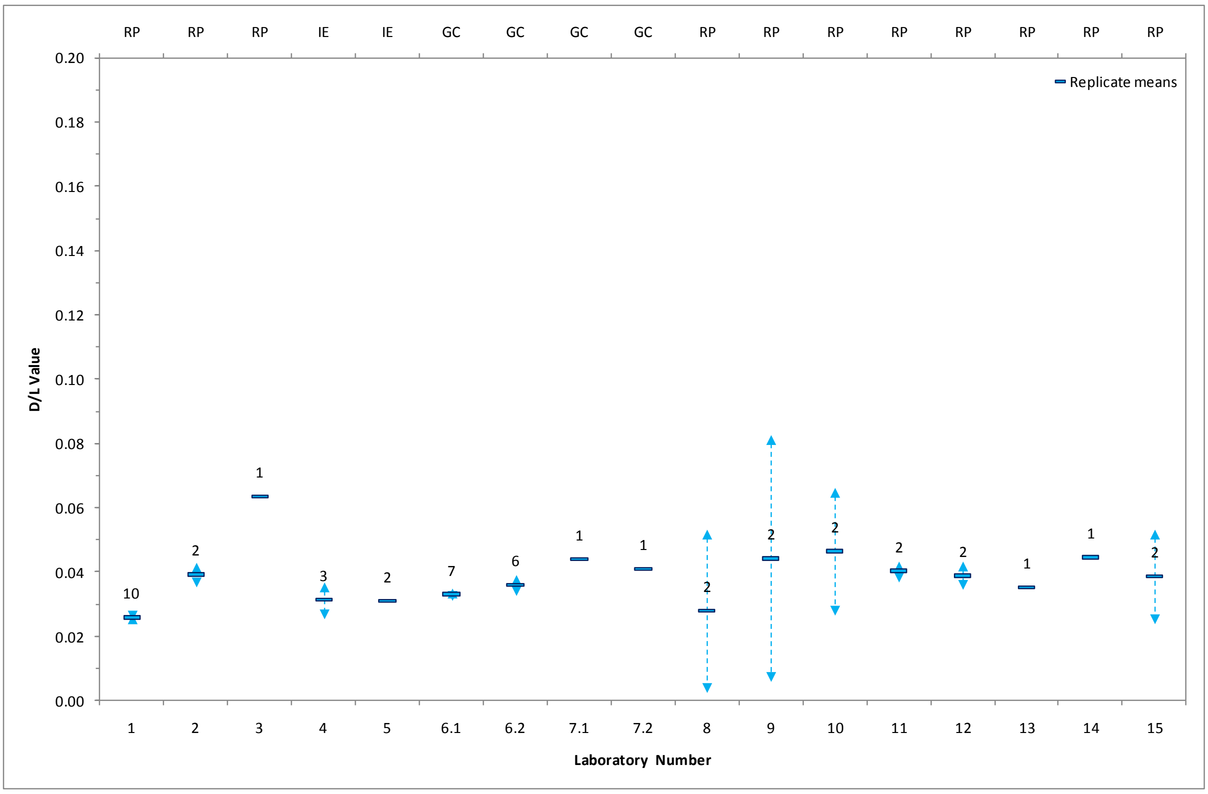


Table 4.26: Summary Statistics for L and D **Leucine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Leu peak area		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	64771	65855	70684	76589	17497	19254	20190	21401	23833	25765	40584	10	25162.0	62.00	7956.9	19.61	39.21	2.262	44.35
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	20364	20225									20294	2	98.2	0.48	69.5	0.34	0.68	12.710	4.35
9	RP	18314	18869									18592	2	392.6	2.11	277.6	1.49	2.99	12.710	18.98
10	RP	10579	11297									10938	2	507.6	4.64	359.0	3.28	6.56	12.710	41.71
11	RP	7605	7470									7537	2	95.4	1.27	67.4	0.89	1.79	12.710	11.37
12	RP	9561	9730									9646	2	119.6	1.24	84.5	0.88	1.75	12.710	11.14
13	RP	6645										6645	1							
14	RP	4973										4973	1							
15	RP	3778	3885									3831	2	75.3	1.97	53.3	1.39	2.78	12.710	17.67
D-Leu peak area		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP	4090	4428	4126	4523	1040	1246	1350	1482	1576	1689	2555	10	1510.3	59.12	477.6	18.69	37.39	2.262	42.29
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	1070	1064									1067	2	4.6	0.43	3.2	0.30	0.61	12.710	3.85
9	RP	1131	1206									1169	2	53.5	4.58	37.8	3.24	6.47	12.710	41.12
10	RP	696	739									717	2	30.3	4.22	21.4	2.99	5.97	12.710	37.94
11	RP	471	447									459	2	16.9	3.69	12.0	2.61	5.21	12.710	33.13
12	RP	595	603									599	2	5.7	0.95	4.0	0.67	1.35	12.710	8.56
13	RP	235										235	1							
14	RP	498										498	1							
15	RP	219	227									223	2	5.3	2.40	3.8	1.70	3.39	12.710	21.55
D+L Leu peak height		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
004	IE	8.483	4.988	4.993								6.155	3	2.0164	32.76	1.1642	18.92	37.83	4.303	81.39
005	IE	5.093	5.079									5.086	2	0.0099	0.19	0.0070	0.14	0.28	12.710	1.75

Table 4.27: Summary Statistics for L and D **Leucine** Concentration Data (pM)

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Leu Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% (k=t_{crit})**
1	RP	3401	3422	3235	3325	3431	3535	3468	3495	3408	3490	3421	10	88.0	2.57	27.8	0.81	1.63	2.262	1.84
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	3344	3307									3326	2	26.0	0.78	18.4	0.55	1.11	12.710	7.04
9	RP	3967	4249									4108	2	199.2	4.85	140.9	3.43	6.86	12.710	43.59
10	RP	4360	4429									4395	2	48.9	1.11	34.6	0.79	1.57	12.710	10.00
11	RP	3812	3827									3819	2	10.8	0.28	7.6	0.20	0.40	12.710	2.53
12	RP	5825	5846									5835	2	14.8	0.25	10.5	0.18	0.36	12.710	2.28
13	RP	5920										5920	1							
14	RP	6720										6720	1							
15	RP	5304	5475									5389	2	121.3	2.25	85.8	1.59	3.18	12.710	20.22
D-Leu Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% (k=t_{crit})**
1	RP	215	230	189	196	204	229	232	242	225	229	219	10	17.3	7.92	5.5	2.50	5.01	2.262	5.66
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP	176	174									175	2	1.3	0.73	0.9	0.51	1.03	12.710	6.53
9	RP	245	272									258	2	18.9	7.31	13.4	5.17	10.34	12.710	65.70
10	RP	287	290									288	2	2.0	0.69	1.4	0.49	0.98	12.710	6.23
11	RP	236	229									233	2	5.0	2.14	3.5	1.51	3.03	12.710	19.24
12	RP	363	362									362	2	0.1	0.03	0.1	0.02	0.05	12.710	0.30
13	RP	210										210	1							
14	RP	673										673	1							
15	RP	308	320									314	2	8.4	2.68	5.9	1.90	3.79	12.710	24.11

Table 4.28: Summary Statistics for L and D **Leucine** D/L Ratio Value

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Leu		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	0.063	0.067	0.058	0.059	0.059	0.065	0.067	0.069	0.066	0.066	0.064	10	0.0038	5.96	0.0012	1.89	3.77	2.262	4.27
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1[1]	GC_A	0.040										0.040	7	0.0010	2.50	0.0004	0.94	1.89	2.447	2.31
6.2[1]	GC_H	0.047										0.047	6	0.0040	8.51	0.0016	3.47	6.95	2.571	8.93
7.1[1]	GC_A	0.044										0.044	1							
7.2[1]	GC_H	0.043										0.043	1							
8	RP	0.053	0.053									0.053	2	0.0000	0.00	0.0000	0.00	0.00	12.710	0.00
9	RP	0.062	0.064									0.063	2	0.0015	2.47	0.0011	1.74	3.49	12.710	22.15
10	RP	0.066	0.065									0.066	2	0.0003	0.42	0.0002	0.30	0.59	12.710	3.77
11	RP	0.062	0.060									0.061	2	0.0015	2.42	0.0010	1.71	3.43	12.710	21.77
12	RP	0.062	0.062									0.062	2	0.0002	0.29	0.0001	0.20	0.41	12.710	2.58
13	RP	0.035										0.035	1							
14	RP	0.100										0.100	1							
15	RP	0.058	0.058									0.058	2	0.0003	0.43	0.0002	0.31	0.61	12.710	3.89

[1]= submitted as the mean and standard deviation of n results.

GC_A = derived using peak area

GC_H = derived using peak height

Figure 4.26: Distribution of D/L Values submitted for Leucine

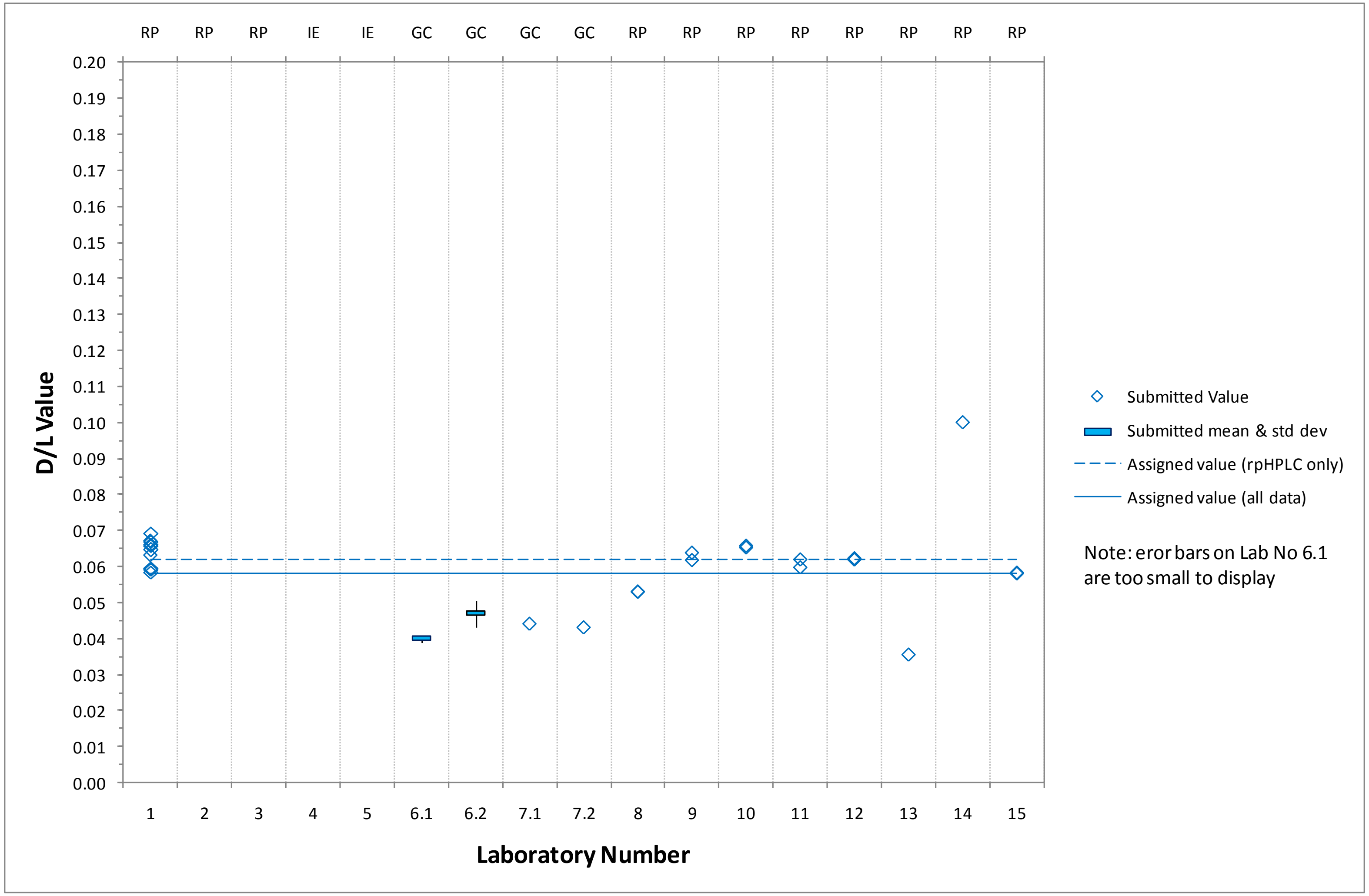


Figure 4.27: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Leucine** (value of n displayed).

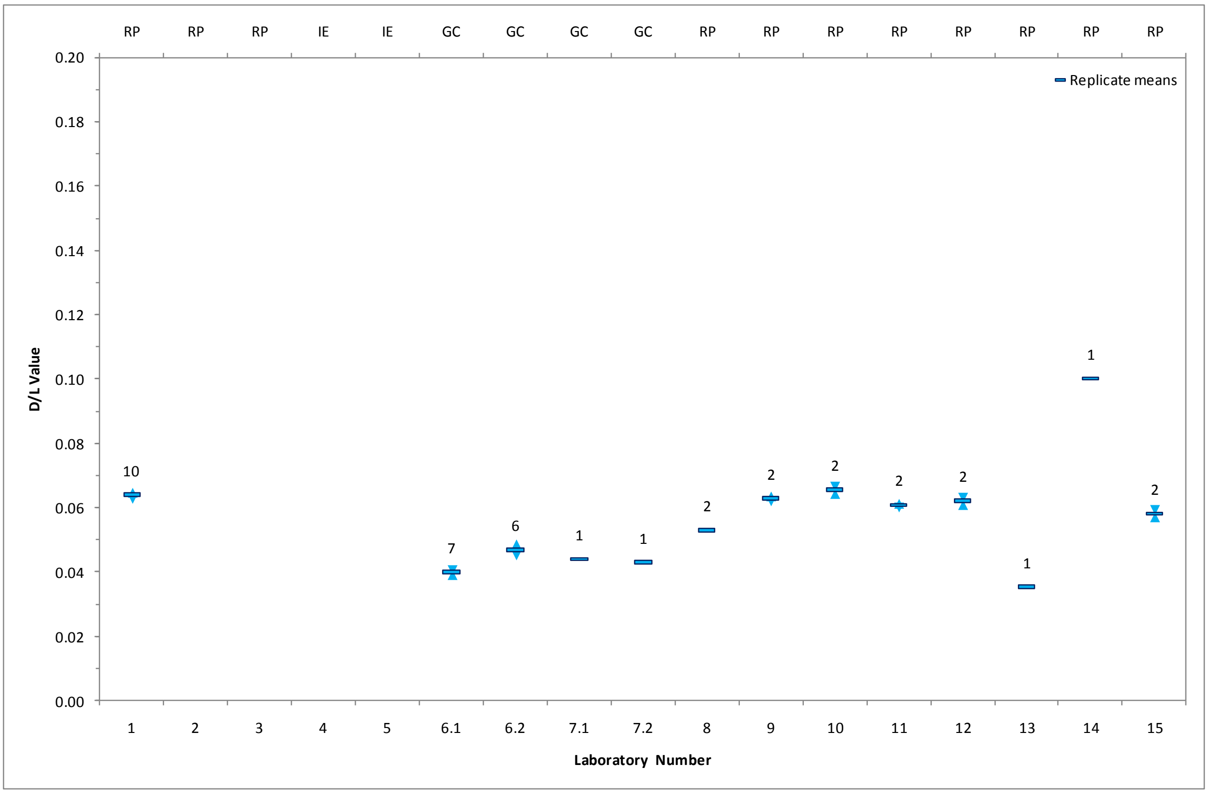


Figure 4.28: Experimental Expanded Uncertainty **(k=t$_{(0.05,df)}$)** of the Mean D/L value for **Leucine** (value of n displayed).

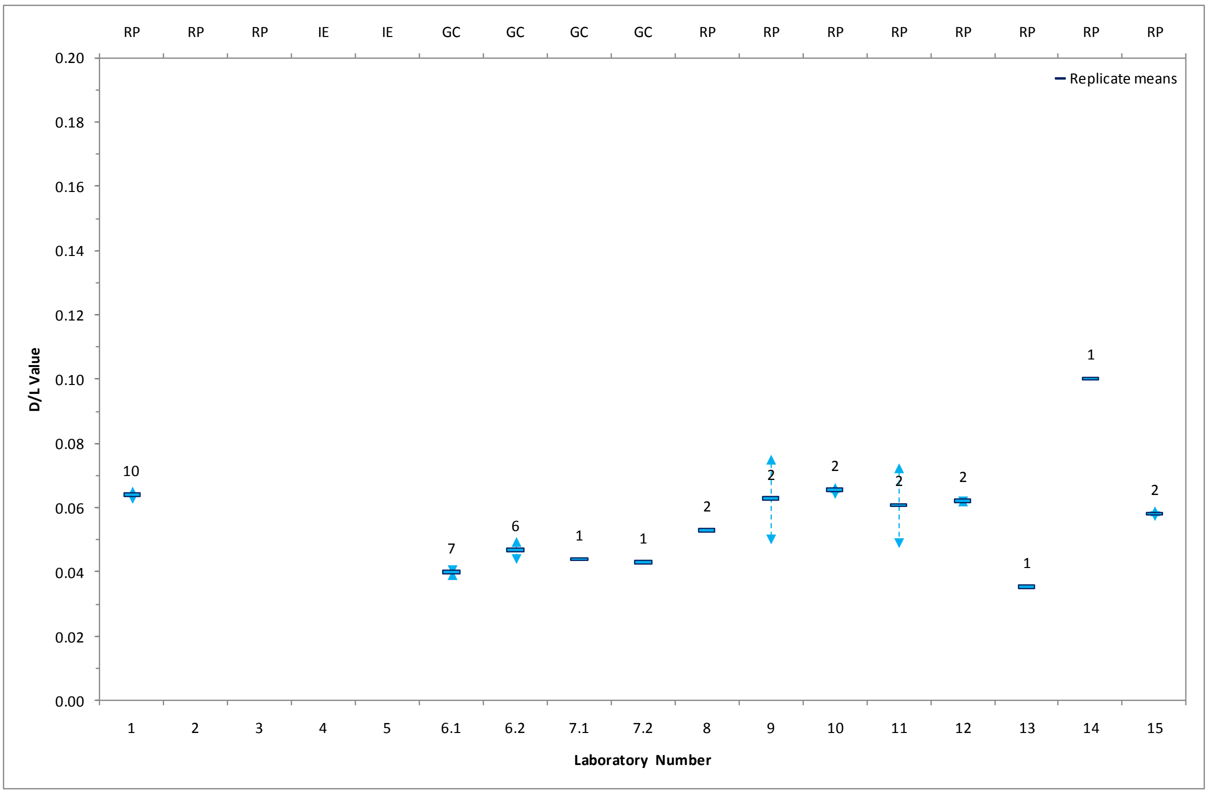


Table 4.29: Summary Statistics for L and D **Tyrosine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Tyr peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP																			
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	9122	9321									9221	2	140.5	1.52	99.4	1.08	2.16	12.710	13.70
10	RP	4903	5214									5058	2	219.7	4.34	155.4	3.07	6.14	12.710	39.04
11	RP	3915	3825									3870	2	63.5	1.64	44.9	1.16	2.32	12.710	14.75
12	RP	4837	4925									4881	2	61.8	1.27	43.7	0.90	1.79	12.710	11.38
13	RP	3175										3175	1							
14	RP	2312										2312	1							
15	RP	1690	1711									1701	2	14.9	0.87	10.5	0.62	1.24	12.710	7.86
D-Tyr peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP																			
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	773	788									780	2	10.9	1.39	7.7	0.98	1.97	12.710	12.51
10	RP	418	433									426	2	10.6	2.49	7.5	1.76	3.52	12.710	22.38
11	RP	297										297	1							
12	RP	376	380									378	2	2.6	0.68	1.8	0.48	0.96	12.710	6.09
13	RP	227										227	1							
14	RP	172										172	1							
15	RP	122	124									123	2	1.7	1.37	1.2	0.97	1.94	12.710	12.30

Table 4.30: Summary Statistics for L and D **Tyrosine** Concentration Data (pM)

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Tyr Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP																			
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	1275	1354									1314	2	56.0	4.26	39.6	3.01	6.03	12.710	38.31
10	RP	1303	1318									1311	2	10.7	0.81	7.6	0.58	1.15	12.710	7.32
11	RP	1266	1264									1265	2	1.2	0.09	0.8	0.07	0.13	12.710	0.85
12	RP	1901	1908									1905	2	5.3	0.28	3.8	0.20	0.40	12.710	2.52
13	RP	1824										1824	1							
14	RP	2015										2015	1							
15	RP	1530	1556									1543	2	17.9	1.16	12.6	0.82	1.64	12.710	10.42
D-Tyr Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP																			
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	108	114									111	2	4.6	4.13	3.2	2.92	5.84	12.710	37.13
10	RP	111	110									110	2	1.1	1.04	0.8	0.73	1.47	12.710	9.34
11	RP	96										96	1							
12	RP	148	147									148	2	0.5	0.31	0.3	0.22	0.44	12.710	2.77
13	RP	130										130	1							
14	RP	150										150	1							
15	RP	110	113									112	2	1.8	1.65	1.3	1.17	2.34	12.710	14.86

Table 4.31: Summary Statistics for L and D **Tyrosine** D/L Ratio Value

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Tyr		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
1	RP																			
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	0.085	0.085									0.085	2	0.0001	0.13	0.0001	0.09	0.19	12.710	1.18
10	RP	0.085	0.083									0.084	2	0.0016	1.85	0.0011	1.31	2.62	12.710	16.66
11	RP	0.076										0.076	1							
12	RP	0.078	0.077									0.078	2	0.0005	0.59	0.0003	0.42	0.83	12.710	5.29
13	RP	0.071										0.071	1							
14	RP	0.074										0.074	1							
15	RP	0.072	0.073									0.072	2	0.0004	0.49	0.0003	0.35	0.70	12.710	4.44

Figure 4.29: Distribution of D/L Values submitted for **Tyrosine**

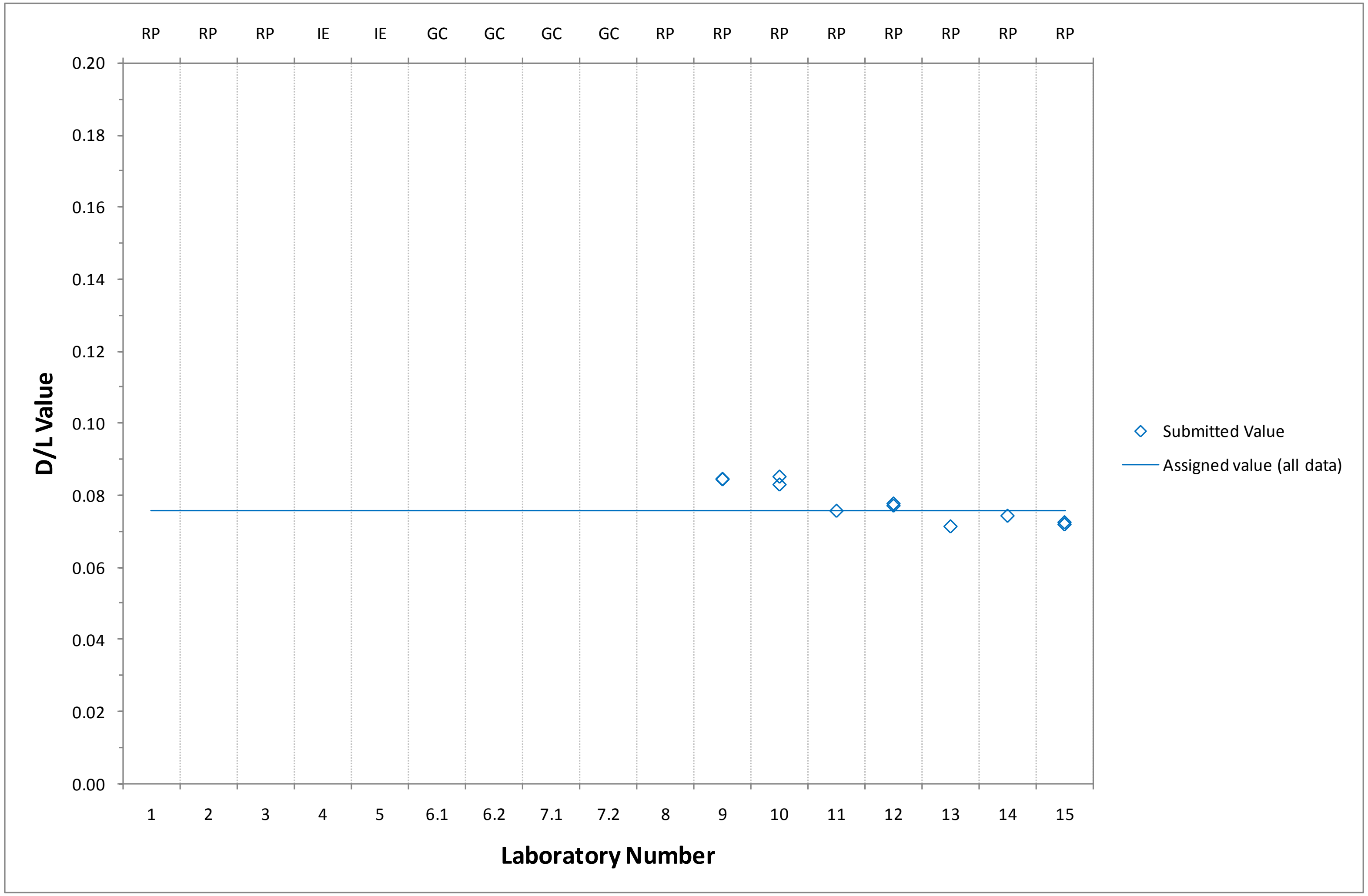


Figure 4.30: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Tyrosine** (value of n displayed).

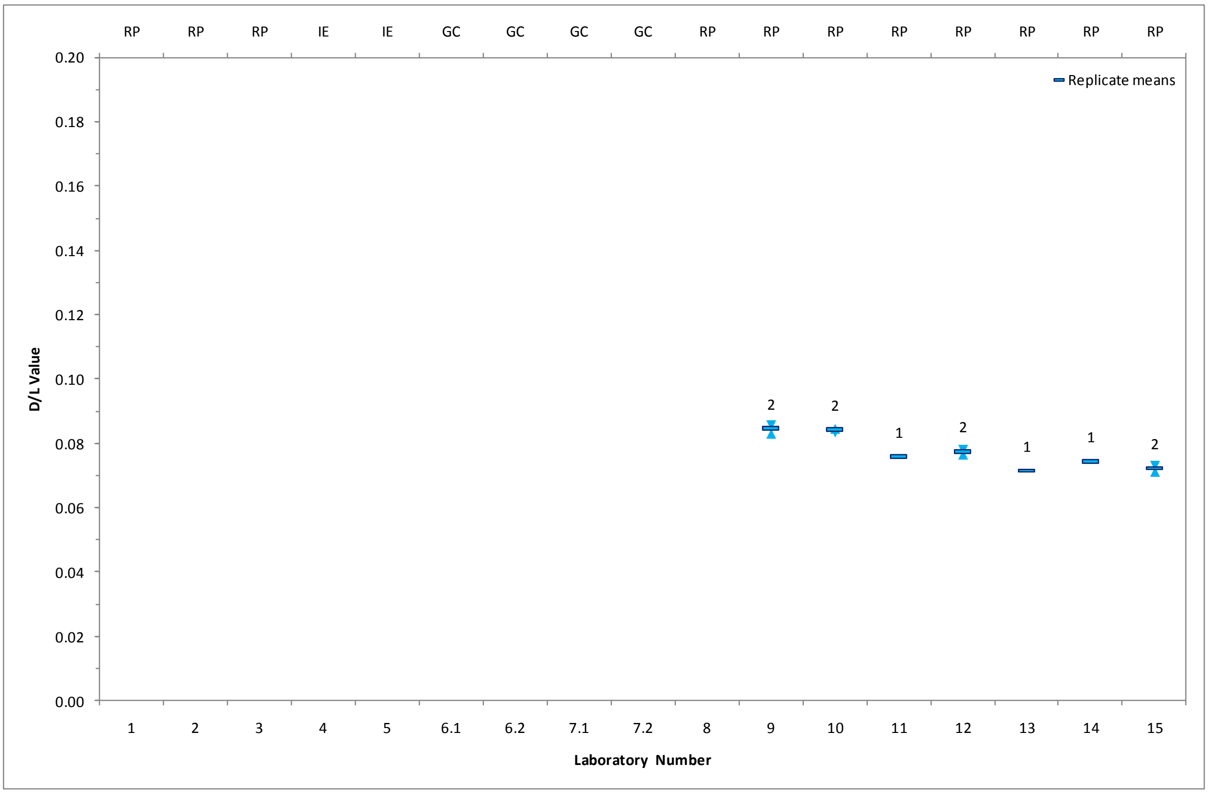


Figure 4.31: Experimental Expanded Uncertainty **(k=$t_{(0.05,df)}$)** of the Mean D/L value for **Tyrosine** (value of n displayed).

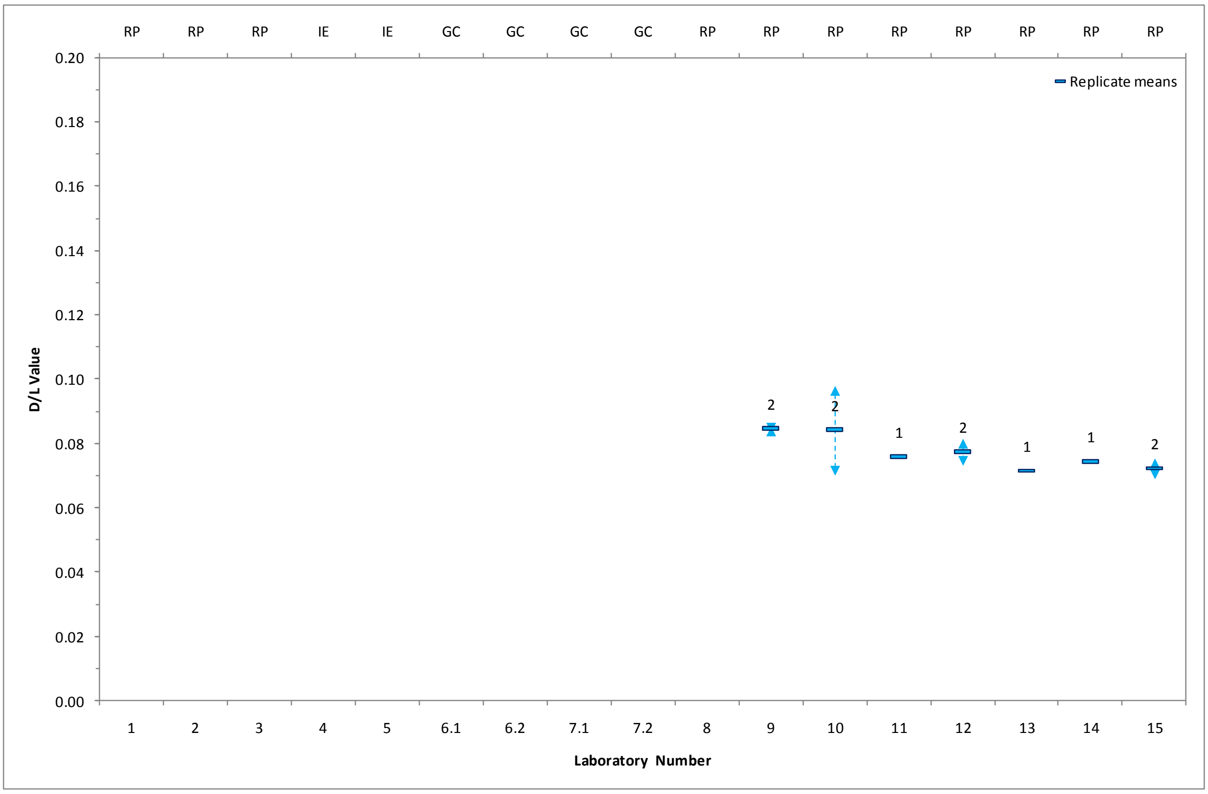


Table 4.32: Summary Statistics for L and D **Methionine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Met peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP																			
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	410.1	399.2									405	2	7.7	1.90	5.4	1.34	69.0	410.1	399.2
10	RP	185.8	207.5									197	2	15.4	7.81	10.9	5.52	138.0	185.8	207.5
11	RP																			
12	RP	235.9	233.7									235	2	1.5	0.65	1.1	0.46	13.7	235.9	233.7
13	RP	783.1										783	1						783.1	
14	RP	644.9										645	1						644.9	
15	RP	46.5	47.4									47	2	0.6	1.37	0.5	0.97	5.8	46.5	47.4
D-Met peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP																			
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	236.5	190.0									213	2	32.8	15.39	23.2	10.88	295.0	236.5	190.0
10	RP	87.1	101.1									94	2	9.9	10.48	7.0	7.41	88.6	87.1	101.1
11	RP																			
12	RP																			
13	RP	114.4										114	1						114.4	
14	RP	81.4										81	1						81.4	
15	RP	27.5	25.5									26	2	1.4	5.39	1.0	3.81	12.8	27.5	25.5

Table 4.33: Summary Statistics for **HPLC Internal Standards**; Peak Area/Height Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-homoArginine peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP	5713	5774	6554	6909	1530	1634	1746	1837	2098	2215	3601	10	2303.1	63.96	728.3	20.22	40.45	2.262	45.75
2	RP	564	548									556	2	11.3	2.03	8.0	1.43	2.87	12.710	18.22
3	RP	336										336	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP	2122	2041									2081	2	57.0	2.74	40.3	1.94	3.87	12.710	24.62
10	RP	1115	1172									1144	2	40.4	3.53	28.5	2.50	4.99	12.710	31.72
11	RP	917	897									907	2	14.0	1.55	9.9	1.09	2.19	12.710	13.90
12	RP	1509	1530									1519	2	15.0	0.99	10.6	0.70	1.39	12.710	8.86
13	RP	1291										1291	1							
14	RP	850										850	1							
15	RP	818	815									817	2	2.3	0.28	1.6	0.20	0.40	12.710	2.56
Norleucine peak height		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
1	RP																			
2	RP																			
3	RP																			
4	IE	0.273	0.100	0.132								0.168	3	0.092	54.68	0.053	31.57	63.14	4.303	135.84
5	IE	0.136	0.132									0.134	2	0.003	2.11	0.002	1.49	2.99	12.710	18.97
6.1	GC																			
6.2	GC																			
7.1	GC																			
7.2	GC																			
8	RP																			
9	RP																			
10	RP																			
11	RP																			
12	RP																			
13	RP																			
14	RP																			
15	RP																			

5 STATISTICAL EVALUATION; *Accuracy & Performance Analysis*

5.1 Background to understanding Performance Evaluation

The purpose of this evaluation is to provide a clear and independent statistical evaluation and comparison of participants' results. In routine analysis a laboratory's evaluation of analytical competence is often restricted to intra-laboratory precision evaluation of repeated analyses or the evaluation of bias using certified reference materials (CRM's). However, in the absence of a suitable, matrix matched CRM with a known value and uncertainty, evaluation of method and/or laboratory bias can be impossible without the cooperation of additional laboratories. Estimations of precision may be excellent when taken in isolation, but may give rise to unrealistically small uncertainties.

5.1.1 z-Scores

Participation in a proficiency test provides the opportunity to evaluate analytical bias by comparing an individual laboratory's result against the assigned value for the test material. Performance is traditionally determined by the calculation of a z-score, calculated using the submitted result, a reference or assigned value and the target value for standard deviation, using a procedure recommended in the IUPAC/ISO/AOAC International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories (Thompson et al., 2006), such that;

$$z = \frac{(\bar{x} - \hat{X})}{\sigma_p}$$

where $\bar{x}$ = the mean of participant's reported replicate results (or simply x for a single reported result)

$\hat{X}$ = the assigned value,

and σ_p = the target standard deviation.

Note that; $(x - \hat{X})$ is the calculation for bias.

Satisfactory performance is indicated by achieving a z-score no greater than 2, i.e.; $|z| \leq 2$.

The results of a typical chemical analysis will be normally distributed about the mean with a known standard deviation. Approximately 95% of data will be expected to lie within 2 standard deviations either side of the mean and 99.7% within ± 3 standard deviations. Thus, it is considered 'satisfactory' if a participant's z-score lies within this range. It follows that if a participant's z-score lies outside $|z| > 2$ there is about a 1 in 20 chance that their result is in fact an acceptable result from the extreme of the distribution. If a participant's z-score lies outside $|z| > 3$ the chance that their result is actually acceptable is only about 1 in 300 (Thompson et al., 2006, ISO 13528, 2005).

5.1.2 The Target Standard Deviation; σ_p

The target standard deviation (σ_p) describes how the data is expected to perform for a given analyte and / or test material and determines the limits of satisfactory performance.

These values are often obtained from collaborative trials as the reproducibility standard deviation ($RSD_R\%$), which describes best practice for a specified method for a given matrix/analyte/ concentration (Thompson et al., 2006).

$$\sigma_p = \frac{RSD_R}{100} \times c$$

where RSD_R = Relative Standard Deviation of Reproducibility from collaborative trial data, expressed as %

and c = concentration, i.e. the assigned value, $\hat{X}$, expressed in relevant units.

In the absence of collaborative trial data, the Horwitz equation (Horwitz et al., 1980, Horwitz, 1982, RSC Analytical Methods Committee, 2004) is widely accepted as a suitable predictive measure for the target standard deviation in chemical analysis. However, the Horwitz function is not necessarily suited to every type of chemical analysis and in the absence of a suitable alternative, the use of perception or fitness-for-purpose criteria may need to be employed, taking into consideration any uncertainty in homogeneity of test materials.

The distribution of submitted results and uncertainty of the assigned value ($u(\hat{X})$) (see section 5.3.1) should be small by comparison to the target standard deviation, (σ_p). This ensures that the data are sufficiently tight to give a measure of confidence in the assigned value, ($\hat{X}$), and that the target value is not overly restrictive.

As a general rule, it can be assumed that participants will be hoping to achieve a satisfactory performance and achieve fitness-for-purpose. It is therefore not an unreasonable expectation that the distribution of submitted results (i.e.; the standard deviation of the assigned value, $\hat{\sigma}$), should be close to the limits of satisfactory performance, σ_p, such that $\hat{\sigma} \approx \sigma_p$. The International Harmonized Protocol (2006) states that if $\hat{\sigma} > 1.2\sigma_p$ then "*laboratories are having difficulty achieving the required reproducibility precision in results from a single population, or that two or more discrepant populations may be represented in the result*".

A further comment is made in the International Harmonised Protocol concerning the uncertainty of the assigned value to ensure it is sufficiently small so as not to overly influence the calculation of z-scores. It is recommended that $u(\hat{X})^2 \leq 0.1\sigma_p{}^2$ which approximates to $u(\hat{X}) \leq 0.3\sigma_p$ as also recommended in ISO 13528 (2005). (Note; The exact value chosen represents the appropriate order of magnitude although the exact value is to some extent discretionary).

5.2 In the absence of Fitness-for-Purpose Criteria

To date, there has not been an inter-laboratory collaborative trial carried out according to international guidelines (AOAC, 2000, Horwitz, 1995) to determine single method precision parameters for amino acid racemization analysis on fossil material. The Horwitz equation requires the measurement units to be expressed as a mass fraction, i.e.; mg/Kg = 10^{-6}, which is not appropriate in the current study as D/L results are expressed as a ratio and are thus dimensionless. Therefore, in the absence of an external value for target standard deviation, it was necessary to use perception using fitness-for-purpose criteria.

The target value chosen during homogeneity evaluation, (σ_h) is an excellent indication of the observed variation within test materials and reflects the uncertainty due to matrix plus the analytical method used for their determination. The relative value of σ_h expressed as a percentage; i.e.; the RSD%, is a more useful value and can be used to set the minimum permissible value for σ_p. Whilst an inter-laboratory collaborative trial reproducibility standard deviation (RSD_R%) would also reflect an additional laboratory component of variation, in the absence of such data, it none the less makes a good starting point for evaluating submitted results and provides a minimum fitness-for-purpose target value.

During the statistical evaluation of data, it was observed that for some amino acids in some test materials provided in this series of studies, the homogeneity target value was too wide compared to the submitted data for the test, suggesting that the **precision between different laboratories in some instances was better than that observed between samples analysed by a single laboratory under repeatability conditions for homogeneity!**

5.2.1 Relative percentage bias

Whilst these observations were surprising, it posed some difficulties in using objective fitness for purpose criteria for the determination of the target values for standard deviation.

In order to overcome this problem and in order to ensure consistency between test materials, in the absence of independently determined performance criteria it was decided to present the data as an assessment of relative bias (%), such that;

$$Relative\ bias\ \% = \frac{(x - \hat{X})}{\hat{X}} \times 100$$

Satisfactory performance was assessed as plus or minus twice the standard deviation of the assigned value, representing 95% confidence limits, i.e.; $\pm 2\hat{\sigma}$.

In this way it was possible to represent participant's results graphically as histograms in a similar way to z-score charts, with the 2 std deviation satisfactory range being given as percentage values rather than ±2.

When calculating z-scores, the use of a standard deviation, σ_p , as the denominator acts to normalize results. This enables performance between different analytes or between different test materials to be compared on a common scale, but requires the target value (σ_p) to be scaled appropriately to the individual analyte or matrix. However, using the assigned value ($\hat{X}$) as the denominator, and calculating the relative percentage bias, still permits a comparison between analytes and test materials but on a common percentage scale, thus providing perhaps a slightly more intuitive presentation of observed bias for individual results.

Laboratory results were calculated from the mean of submitted replicate data so as not to dominate and unfairly influence the distribution by a single method, analyst or single test material. The distributions of the mean values are presented as dot plots in Figure 5.1. On this occasion, performance has not been determined by the calculation of z-scores but rather an evaluation of bias has been carried out. Laboratory mean values and relative percentage bias for each amino acid are given in Table 5.1. and shown as histograms in Figures 5.2 – 5.18.

5.3 The Assigned Value, $\hat{X}$

The reference or assigned value, $\hat{X}$, is the best estimate of the true concentration of each analyte. Depending on the nature of a test material, this can be done in a number of different ways, for example the use of a reference value from a Certified Reference Material, a consensus of expert laboratories, or the consensus of submitted results.

In determining the assigned value for a specific analyte, the robust mean is often used as the best estimate in a large data set as it minimises the effect of outliers and gives a fairer estimate of central tendency. However, for small data sets such as here, whilst the robust mean may still be preferable to the standard mean, the influence of extreme values may still be significant. In such instances, the use of the median may be more suitable or even the mode.

5.3.1 The uncertainty of the Assigned value $u(\hat{X})$.

When determining the appropriate measure of central tendency, the effect of the uncertainty of the assigned value ($u(\hat{X})$) on performance assessment also needs to be given consideration. If there is too much uncertainty associated with the assigned value, i.e.; either *m* is too small or the distribution of results is too large, then this can have an adverse impact by exaggerating observed bias. For the robust mean and median:

$$u(\hat{X}) = \frac{\hat{\sigma}}{\sqrt{m}}$$

Where m = the number of laboratory results used to calculate the robust mean or median

and $\hat{\sigma}$ = the standard deviation of the robust mean or median absolute deviation (sMAD). (Note this is not the same as the target standard deviation used for calculating z-scores (σ_p)).

For the mode, $u(\hat{X})$ is taken to be directly equivalent to the standard error of the mode, (SEM).

5.4 Derivation of $\hat{X}$ for Amino Acids in Ostrich Egg Shell (A) Test Material

In this study all assigned values have been determined as the consensus of submitted data, which due to the low numbers of participants involved, equates to the consensus from expert laboratories!

Whilst assessing the data, in many cases it became clear that the robust mean (Ellison, 2002b, RSC Analytical Methods Committee, 1989, RSC Analytical Methods Committee, 2001) was strongly influenced by extreme values resulting in a skewed distribution with a high or low end tail. This appeared largely influenced by method and on occasions by an individual laboratory where more than one result was submitted using the same method, but carried out using a different instrument or analyst. In addition, when determining the mode (Ellison, 2002a, RSC Analytical Methods Committee, 2006, Lowthian and Thompson, 2002), it became clear that due to the low numbers of results, additional modes were identified due to only a couple of values and in some cases only a single data point. Plots showing the modal distributions derived using the kernel density Excel add-in (Ellison, 2002a) are shown against each histogram for amino acids with eight or more data points.In cases where there were two evenly matched modes or where a smaller second mode was predominated by data using a specific method such as GC, it would not be appropriate to penalise these laboratories by comparison against an assigned value determined from the primary or first mode. There is no judgment being made as to which set of results is '*correct*', therefore, it would not be appropriate to calculate performance for GC results using an assigned value determined from HPLC values if the GC data clustered differently. In situations such as this where the method may be empirical, the mode should not be used. Regrettably submitted results by GC were limited making it difficult to know whether the observed differences are genuine method differences or simply extreme values.

For these reasons, the median has been used as the most appropriate measure of central tendency for all amino acids. The median ignores the effect of outliers and assumes a normal distribution placing data symmetrically placed either side of the mid-point. This allows for any asymmetry arising from bimodality to be seen in the histograms but makes no judgment as to the correct mode.

Proficiency tests in principle tend not to be method prescriptive unless methods are known to be empirical and produce different results. The extent of any such differences between GC and HPLC or even between rpHPLC and HPLC-IE for the analysis of amino acid racemization, have not been fully established to date. Therefore, in this proficiency test, GC data have been included with HPLC values and initially evaluated against the same assigned value.

However, where GC data has been provided, for aspartic acid/asparagine, alanine, valine and phenylalanine, GC data can be seen to contribute to high or low end tails. Whilst in this test material GC results for glutamic acid/ glutamine, alloisoleucine/isoleucine and leucine appear to fall within the general distribution of the data, for consistency with other test materials in this series, rpHPLC results have also been evaluated separately for comparison. Insufficient data prevented a separate evaluation for GC or HPLC-IE methods individually.

The medians used to set the assigned values for all amino acids, together with the number of laboratory results *m,* the standard deviation of the assigned value*,* $\hat{\sigma}$ and the standard uncertainty of the assigned value, $u(\hat{X})$, are given in Table 5.2. Table 5.3 then gives the percentage of laboratories with mean values falling within ± 2 standard deviations of the assigned value.

5.5 Interpreting Results - a word of caution.

Caution should be exercised when evaluating the results from this study. Whilst every effort has been made to provide a statistically sound and informative comparison and assessment of data, results from all statistical evaluations should be treated for information only due to the absence of external reference data and the uncertainty surrounding assessment parameters.

The report indicates a number of issues such as the level of agreement between HPLC and GC or even between reverse phase HPLC and ion-exchange HPLC methods, and whether these approaches should be considered empirical, such that the method defines the output. This is suggested from results of a number of amino acids. A greater number of laboratories submitting GC data may have helped to answer this. Determination of method specific assigned values would therefore provide truer estimates of bias and uncertainty and a more accurate performance evaluation.

Obtaining an independent and externally derived precision estimate for the target standard deviation such as the reproducibility standard deviation obtained from a collaborative trial becomes paramount for the future. As an indicator of best practice this would provide guideline uncertainty estimates (so long as a laboratory's repeatability complied with published values), define reference values for the use of any remaining material in place of CRMs enhancing quality control processes, and permit the objective assessment of participants' PT data in future studies.

Table 5.1: Results and Relative Percentage Bias for Total Hydrolysed Amino Acids in OES (A) Test Material

Lab No.	method	Total Hydrolysed Amino Acid (THAA)							
		Asx D/L (all)		Asx D/L (rpHPLC)		Glx D/L (all)		Glx D/L (rpHPLC)	
		assigned value	0.379	assigned value	0.370	assigned value	0.087	assigned value	0.088
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
1	RP	0.350	-7.6	0.350	-5.4	0.078	-9.9	0.078	-11.5
2	RP	0.349	-7.8	0.349	-5.6	0.069	-20.0	0.069	-21.5
3	RP	0.365	-3.6	0.365	-1.3	0.075	-13.2	0.075	-14.8
4	IE								
5	IE								
6.1	GC	0.379	0.1			0.082	-5.6		
6.2	GC	0.453	19.6			0.105	20.9		
7.1	GC	0.393	3.8			0.086	-1.0		
7.2	GC	0.427	12.7			0.086	-1.0		
8	RP	0.361	-4.8	0.361	-2.5	0.085	-2.2	0.085	-3.9
9	RP	0.380	0.3	0.380	2.7	0.095	9.2	0.095	7.2
10	RP	0.382	0.9	0.382	3.3	0.094	8.4	0.094	6.5
11	RP	0.379	0.0	0.379	2.4	0.092	5.5	0.092	3.6
12	RP	0.385	1.5	0.385	4.0	0.094	8.7	0.094	6.8
13	RP	0.369	-2.6	0.369	-0.3	0.087	0.0	0.087	-1.8
14	RP	0.377	-0.4	0.377	2.0	0.091	5.2	0.091	3.3
15	RP	0.370	-2.3	0.370	0.0	0.088	1.9	0.088	0.0

Results shown are the average of replicate values where more than one value was given, or as submitted by participants, where a mean value was provided.

Table 5.1: Results and Relative Percentage Bias for Total Hydrolysed Amino Acids in OES (A) Test Material (continued)

Lab No.	method	Total Hydrolysed Amino Acid (THAA)							
		Ser D/L (rpHPLC)		Arg D/L (rpHPLC)		Ala D/L		Ala D/L (rpHPLC)	
		assigned value	0.326	assigned value	0.134	assigned value	0.092	assigned value	0.095
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
1	RP	0.329	0.6			0.094	2.3	0.094	-1.0
2	RP	0.326	-0.3	0.125	-6.6	0.084	-8.4	0.084	-11.4
3	RP	0.356	9.0	0.184	37.3	0.108	17.1	0.108	13.3
4	IE								
5	IE								
6.1	GC					0.077	-16.4		
6.2	GC					0.072	-21.8		
7.1	GC					0.077	-16.4		
7.2	GC								
8	RP	0.345	5.7			0.090	-2.3	0.090	-5.5
9	RP	0.326	-0.2	0.160	19.5	0.095	3.5	0.095	0.1
10	RP	0.332	1.8	0.153	14.2	0.098	6.9	0.098	3.4
11	RP	0.325	-0.5	0.126	-5.9	0.095	3.4	0.095	0.0
12	RP	0.329	0.9	0.122	-8.9	0.104	12.8	0.104	9.2
13	RP	0.319	-2.2	0.124	-7.8	0.085	-8.1	0.085	-11.1
14	RP	0.326	0.0	0.134	0.0	0.102	10.5	0.102	6.9
15	RP	0.318	-2.5	0.135	0.4	0.088	-4.6	0.088	-7.7

Results shown are the average of replicate values where more than one value was given, or as submitted by participants, where a mean value was provided.

Table 5.1: Results and Relative Percentage Bias for Total Hydrolysed Amino Acids in OES (A) Test Material (continued)

Lab No.	method	Total Hydrolysed Amino Acid (THAA)							
		Val D/L		Val D/L (rpHPLC)		Phe D/L		Phe D/L (rpHPLC)	
		assigned value	0.029	assigned value	0.030	assigned value	0.079	assigned value	0.079
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
1	RP	0.027	-9.5	0.027	-10.4	0.074	-6.5	0.074	-7.0
2	RP	0.030	1.0	0.030	0.0	0.071	-10.5	0.071	-11.0
3	RP	0.033	11.0	0.033	9.9	0.080	1.4	0.080	0.8
4	IE								
5	IE								
6.1	GC	0.019	-35.3			0.067	-15.2		
6.2	GC	0.019	-35.3			0.090	13.9		
7.1	GC	0.030	2.2			0.064	-19.0		
7.2	GC	0.022	-25.0			0.067	-15.2		
8	RP	0.032	7.3	0.032	6.2	0.082	3.1	0.082	2.5
9	RP	0.032	8.1	0.032	7.0	0.082	3.5	0.082	2.9
10	RP	0.033	11.1	0.033	10.0	0.083	5.0	0.083	4.4
11	RP	0.029	0.0	0.029	-1.0	0.079	0.6	0.079	0.0
12	RP	0.027	-8.0	0.027	-8.9	0.081	2.7	0.081	2.1
13	RP	0.023	-20.6	0.023	-21.4	0.078	-0.8	0.078	-1.3
14	RP	0.036	22.7	0.036	21.4	0.078	-1.2	0.078	-1.8
15	RP	0.029	-2.7	0.029	-3.6	0.079	0.0	0.079	-0.6

Results shown are the average of replicate values where more than one value was given, or as submitted by participants, where a mean value was provided.

Table 5.1: Results and Relative Percentage Bias for Total Hydrolysed Amino Acids in OES (A) Test Material (continued)

Lab No.	method	Total Hydrolysed Amino Acid (THAA)							
		D-Aile/L-Ile (all)		D-Aile/L-Ile (rpHPLC)		Leu D/L (all)		Leu D/L (rpHPLC)	
		assigned value	0.039	assigned value	0.039	assigned value	0.058	assigned value	0.062
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
1	RP	0.026	-33.3	0.026	-33.8	0.064	9.9	0.064	3.0
2	RP	0.039	0.9	0.039	0.0				
3	RP	0.063	63.1	0.063	61.7				
4	IE	0.031	-19.5						
5	IE	0.031	-20.3						
6.1	GC	0.033	-15.2			0.040	-31.3		
6.2	GC	0.036	-7.5			0.047	-19.2		
7.1	GC	0.044	13.1			0.044	-24.4		
7.2	GC	0.041	5.4			0.043	-26.1		
8	RP	0.028	-28.0	0.028	-28.7	0.053	-8.9	0.053	-14.7
9	RP	0.044	13.7	0.044	12.8	0.063	8.0	0.063	1.2
10	RP	0.046	19.3	0.046	18.3	0.066	12.7	0.066	5.6
11	RP	0.040	3.5	0.040	2.6	0.061	4.6	0.061	-2.0
12	RP	0.039	0.0	0.039	-0.9	0.062	6.8	0.062	0.0
13	RP	0.035	-9.4	0.035	-10.2	0.035	-39.2	0.035	-43.0
14	RP	0.045	14.6	0.045	13.6	0.100	72.1	0.100	61.2
15	RP	0.039	-0.5	0.039	-1.4	0.058	0.0	0.058	-6.3

Results shown are the average of replicate values where more than one value was given, or as submitted by participants, where a mean value was provided.

Table 5.1: Results and Relative Percentage Bias for Total Hydrolysed Amino Acids in OES (A) Test Material (continued)

Lab No.	method	Total Hydrolysed Amino Acid (THAA)	
		Tyr D/L (rpHPLC)	
		assigned value	0.076
		result D/L	relative bias %
1	RP		
2	RP		
3	RP		
4	IE		
5	IE		
6.1	GC		
6.2	GC		
7.1	GC		
7.2	GC		
8	RP		
9	RP	0.085	11.6
10	RP	0.084	11.0
11	RP	0.076	0.0
12	RP	0.078	2.2
13	RP	0.071	-5.7
14	RP	0.074	-1.8
15	RP	0.072	-4.6

Results shown are the average of replicate values where more than one value was given, or as submitted by participants, where a mean value was provided.

Table 5.2: Assigned Values, Standard Deviations and Standard Uncertainties

analyte	assigned value					
	m	Median ($\hat{X}$)	sMAD ($\hat{\sigma}$)	RSD %	Std uncertainty of median ($u(\hat{X})$)	RSU %
Asx D/L (all[a])	15	0.379	0.015	3.84	0.0038	0.99
Asx D/L (rpHPLC)	11	0.370	0.014	3.76	0.0042	1.13
Glx D/L (all[a])	15	0.087	0.007	8.32	0.0019	2.15
Glx D/L (rpHPLC)	11	0.088	0.011	12.72	0.0034	3.83
Ser D/L (rpHPLC)	11	0.326	0.004	1.27	0.0012	0.38
Arg D/L (rpHPLC)	9	0.134	0.015	11.55	0.0052	3.85
Ala D/L (all[a])	14	0.092	0.011	12.25	0.0030	3.27
Ala D/L (rpHPLC)	11	0.095	0.010	10.24	0.0029	3.09
Val D/L (all[a])	15	0.029	0.004	14.13	0.0011	3.65
Val D/L (rpHPLC)	11	0.030	0.004	13.23	0.0012	3.99
Phe D/L (all[a])	15	0.079	0.004	5.14	0.0010	1.33
Phe D/L (rpHPLC)	11	0.079	0.003	3.12	0.0007	0.94
D-Aile/L-Ile (all[b])	17	0.039	0.008	20.37	0.0019	4.94
D-Aile/L-Ile (rpHPLC)	11	0.039	0.007	18.92	0.0022	5.71
Leu D/L (all[a])	13	0.058	0.011	18.81	0.0030	5.22
Leu D/L (rpHPLC)	9	0.062	0.005	8.24	0.0017	2.75
Tyr D/L (rpHPLC)	7	0.076	0.005	6.89	0.0020	2.61

[a] = rpHPLC and GC data [b] = rpHPLC, GC and HPLC-IE data
m = number of replicate mean values sMAD = median absolute deviation
RSD% = Relative standard deviation expressed as a percentage
RSU% = Relative standard uncertainty expressed as a percentage

Table 5.3: Satisfactory Performance(Percentage within 95% Confidence Interval)

analyte	assigned value			
	Median ($\hat{X}$)	Satisfactory m	Total number of m	Percent satisfactory
Asx D/L (all[a])	0.379	12	15	80%
Asx D/L (rpHPLC)	0.370	11	11	100%
Glx D/L (all[a])	0.087	13	15	87%
Glx D/L (rpHPLC)	0.088	11	11	100%
Ser D/L (rpHPLC)	0.326	8	11	73%
Arg D/L (rpHPLC)	0.134	8	9	89%
Ala D/L (all[a])	0.092	14	14	100%
Ala D/L (rpHPLC)	0.095	11	11	100%
Val D/L (all[a])	0.029	13	15	87%
Val D/L (rpHPLC)	0.030	11	11	100%
Phe D/L (all[a])	0.079	10	15	67%
Phe D/L (rpHPLC)	0.079	9	11	82%
D-Aile/L-Ile (all[b])	0.039	16	17	94%
D-Aile/L-Ile (rpHPLC)	0.039	10	11	91%
Leu D/L (all[a])	0.058	11	13	85%
Leu D/L (rpHPLC)	0.062	7	9	78%
Tyr D/L (rpHPLC)	0.076	7	7	100%

[a] = rpHPLC and GC data [b] = rpHPLC, GC and HPLC-IE data m = number of participants' results

Figure 5.1: Distribution of Participants' Average Measurement Values

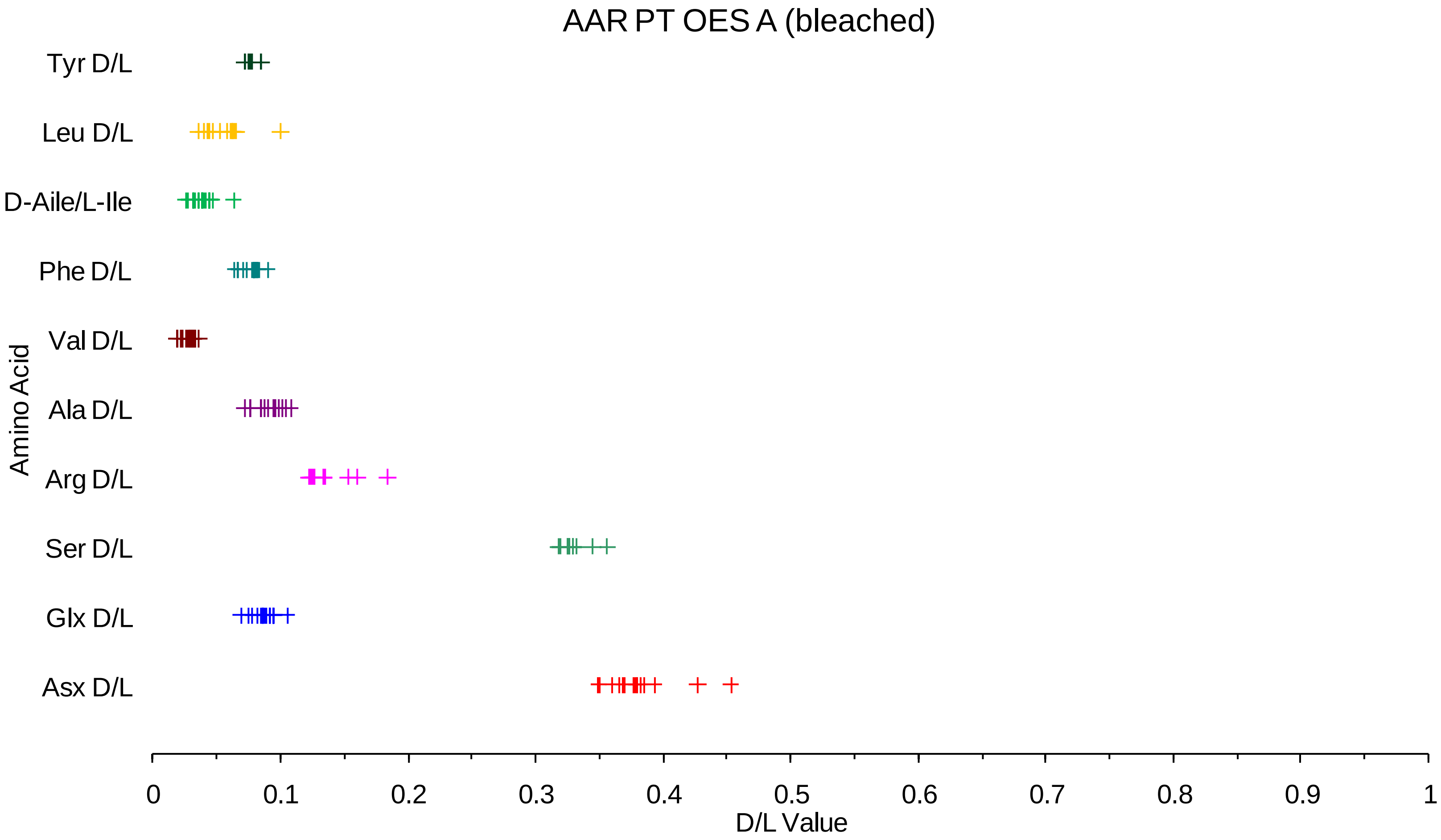


Figure 5.2: Relative Percentage Bias for **Aspartic Acid / Asparagine D/L Results (all data)** in Ostrich Egg Shell (A) Test Material

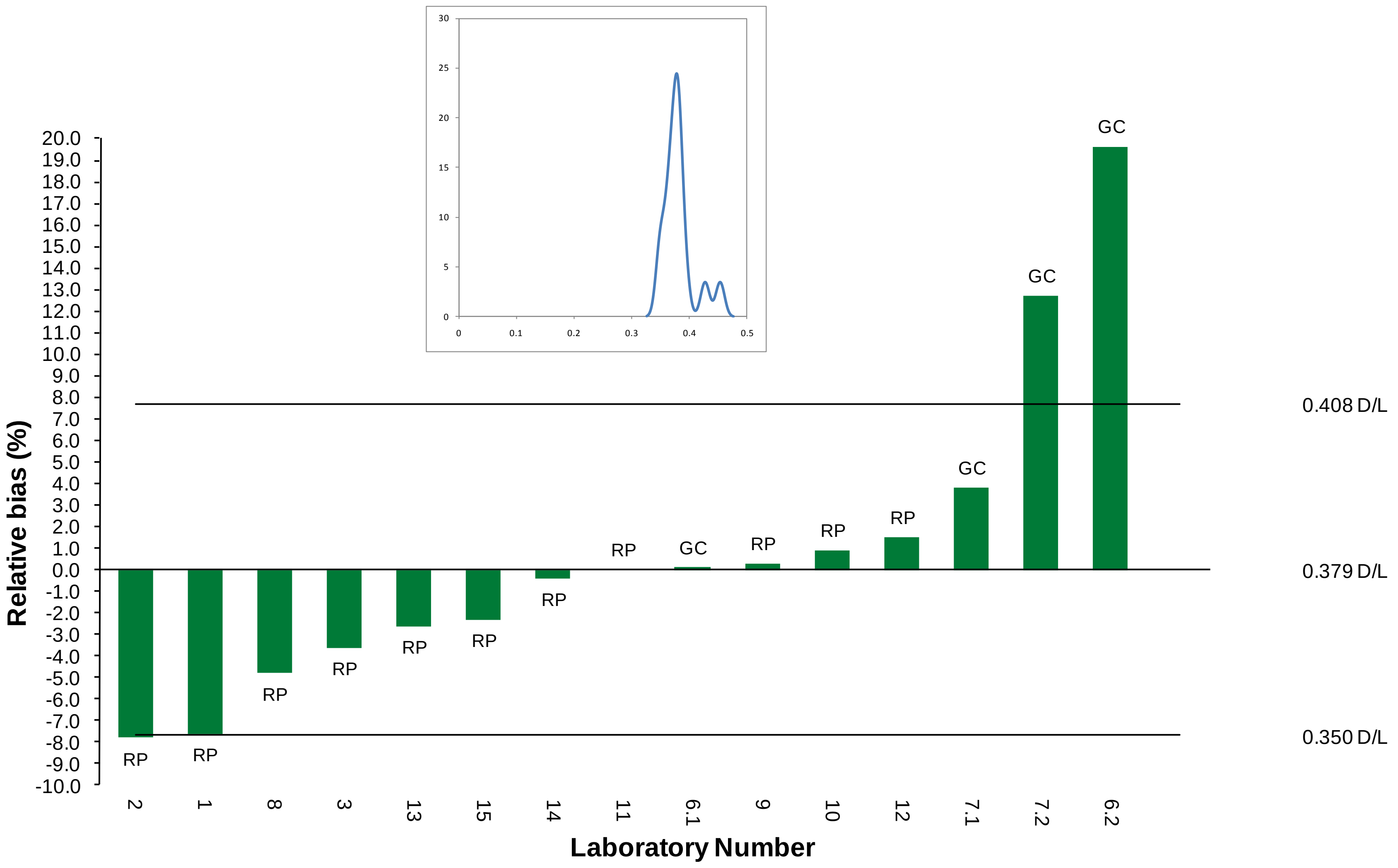


Figure 5.3: Relative Percentage Bias for **Aspartic Acid / Asparagine D/L Results (rpHPLC data only)** in Ostrich Egg Shell (A) Test Material

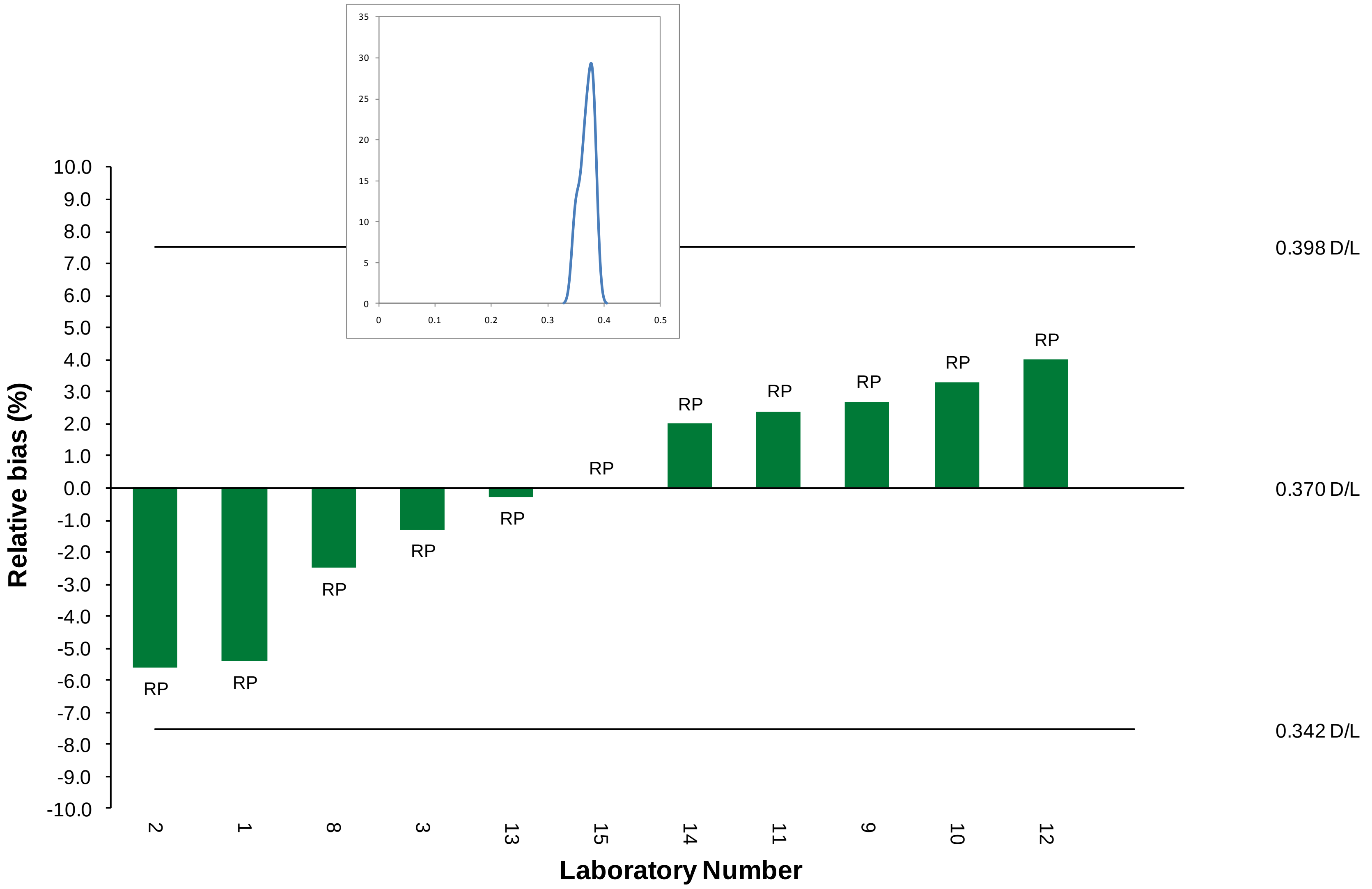


Figure 5.4: Relative Percentage Bias for **Glutamic Acid / Glutamate D/L Results (all data)** in Ostrich Egg Shell (A) Test Material

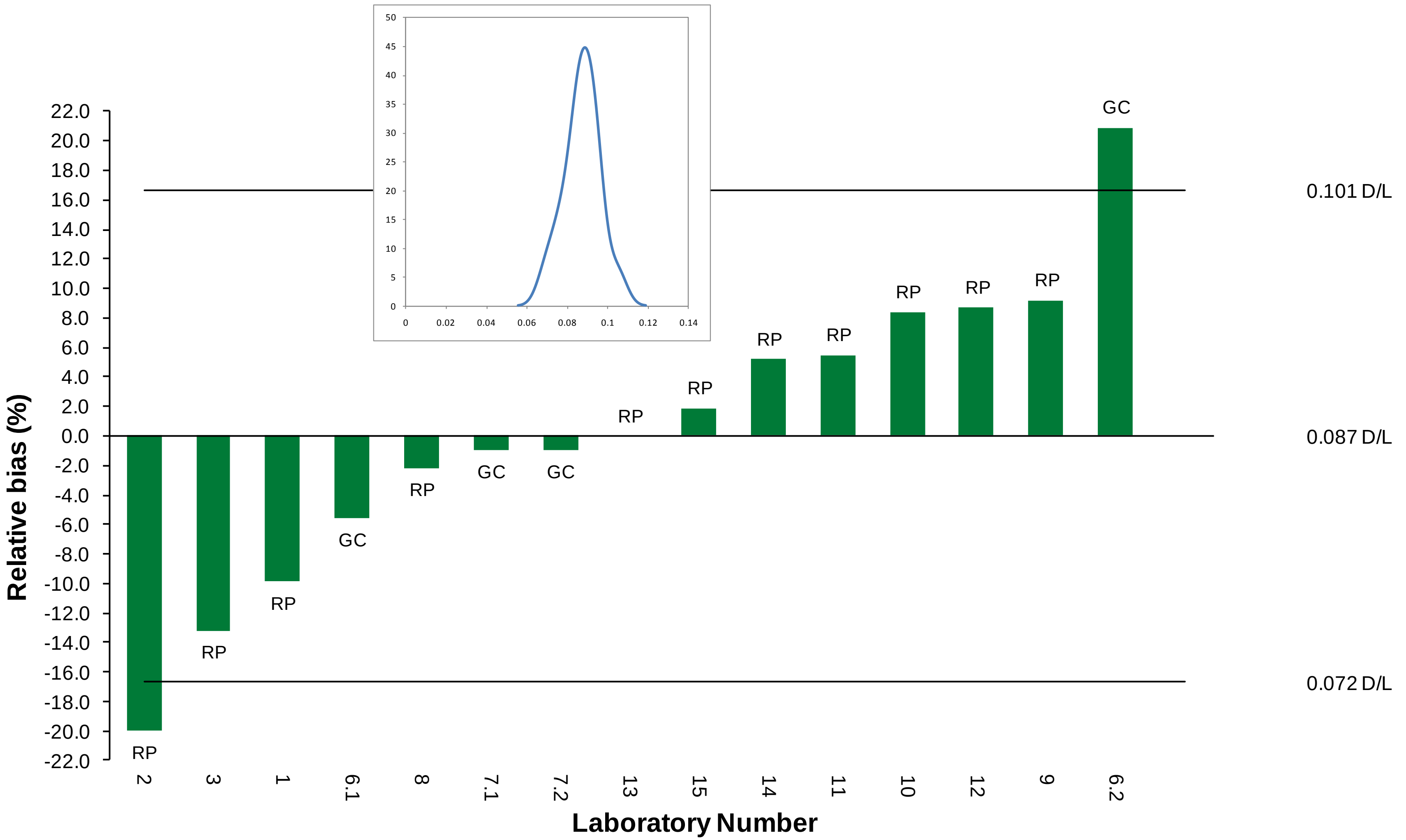


Figure 5.5: Relative Percentage Bias for **Glutamic Acid / Glutamate D/L Results (rpHPLC data only)** in Ostrich Egg Shell (A) Test Material

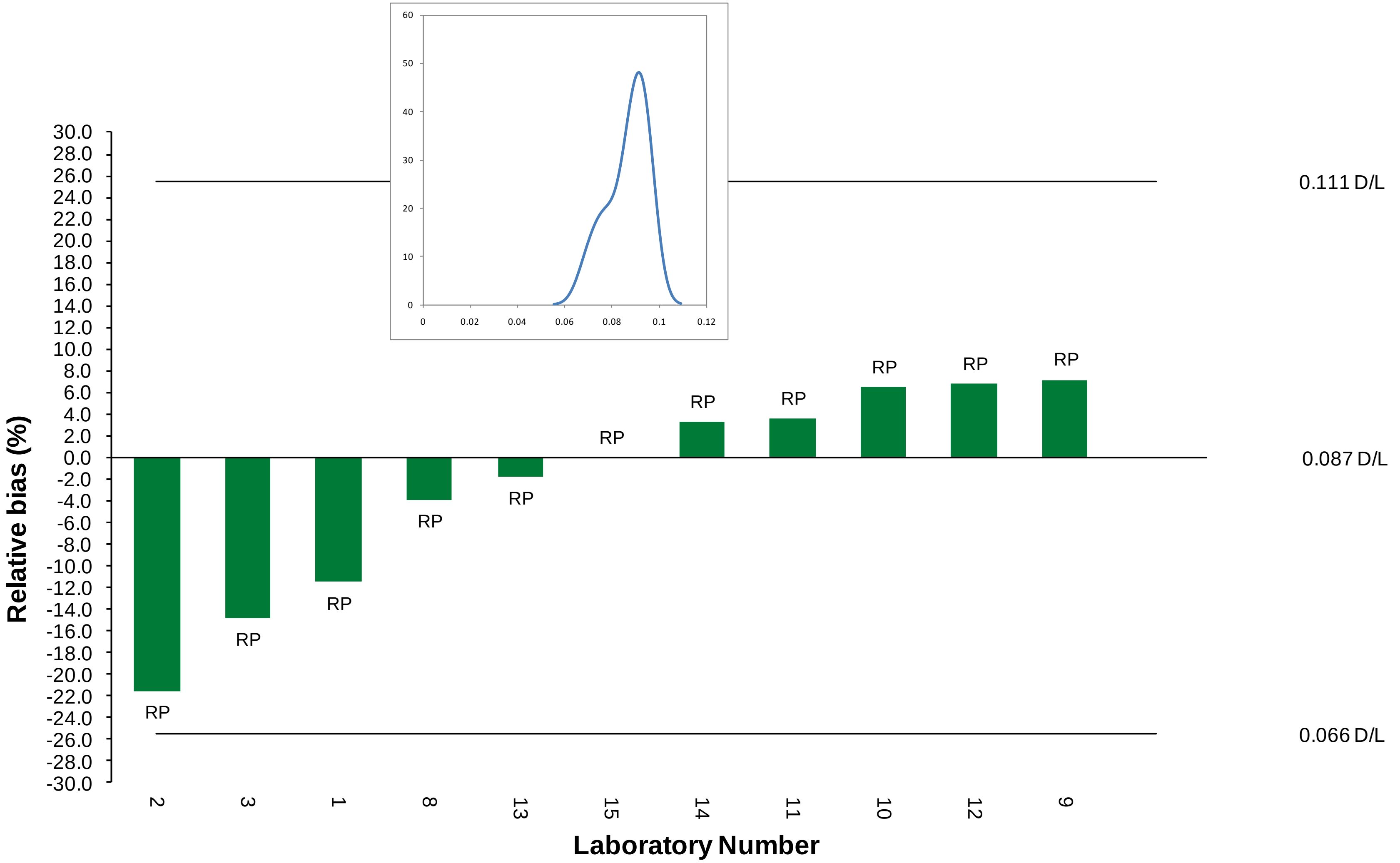


Figure 5.6: Relative Percentage Bias for **Serine D/L Results (all / rpHPLC data)** in Ostrich Egg Shell (A) Test Material

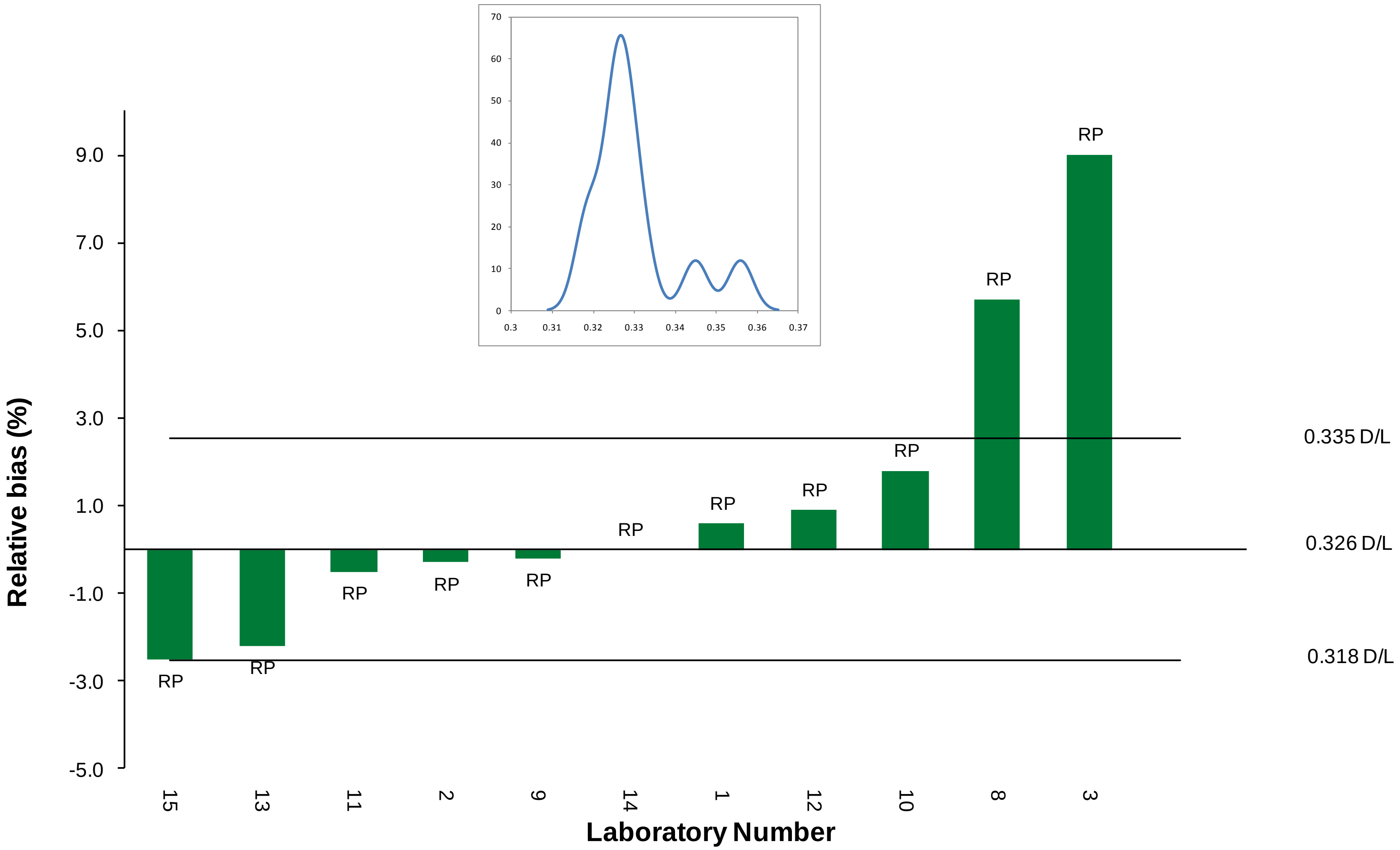


Figure 5.7: Relative Percentage Bias for **Arginine D/L Results (rpHPLC data only)** in Ostrich Egg Shell (A) Test Material

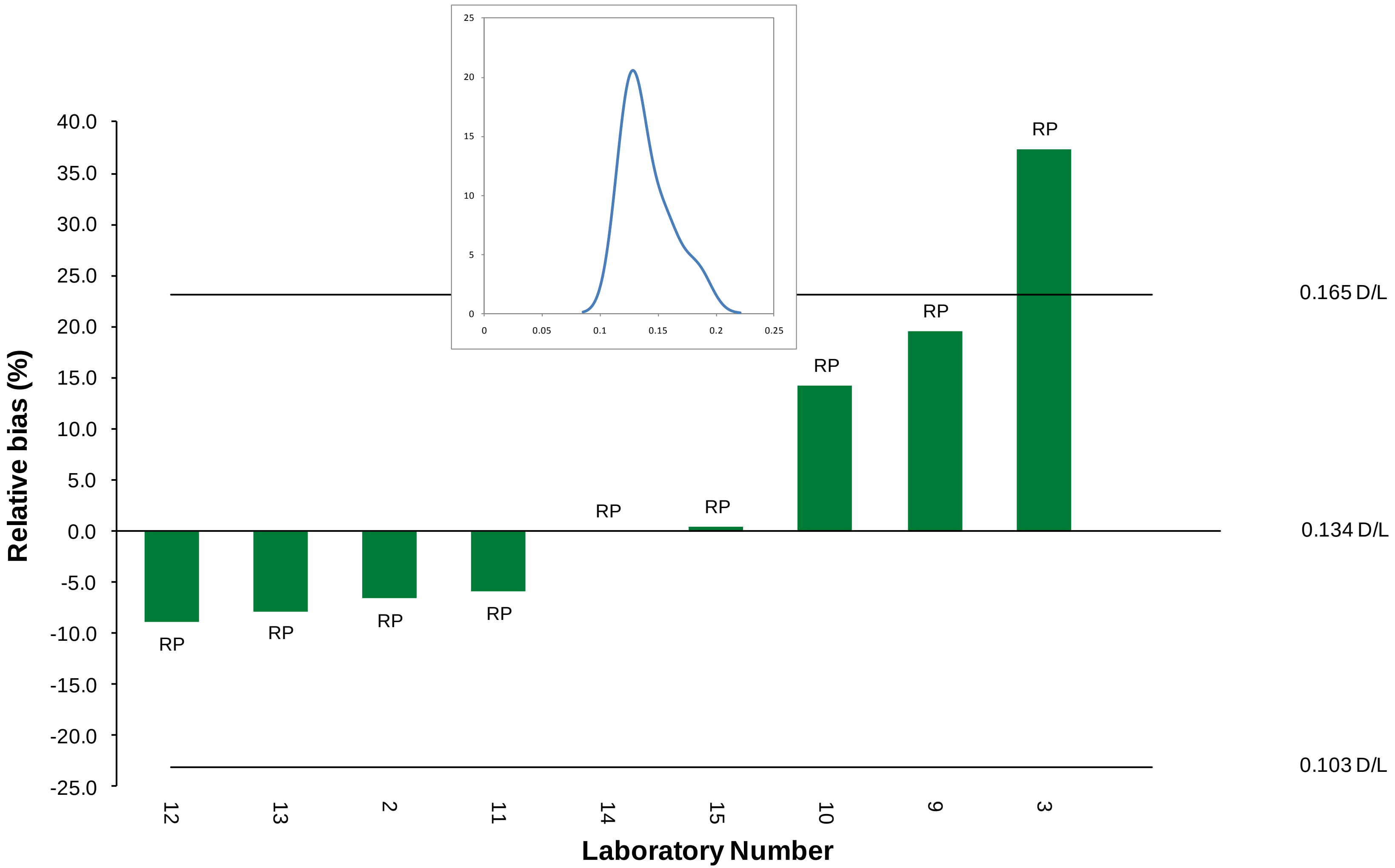


Figure 5.8: Relative Percentage Bias for **Alanine D/L Results (all data)** in Ostrich Egg Shell (A) Test Material

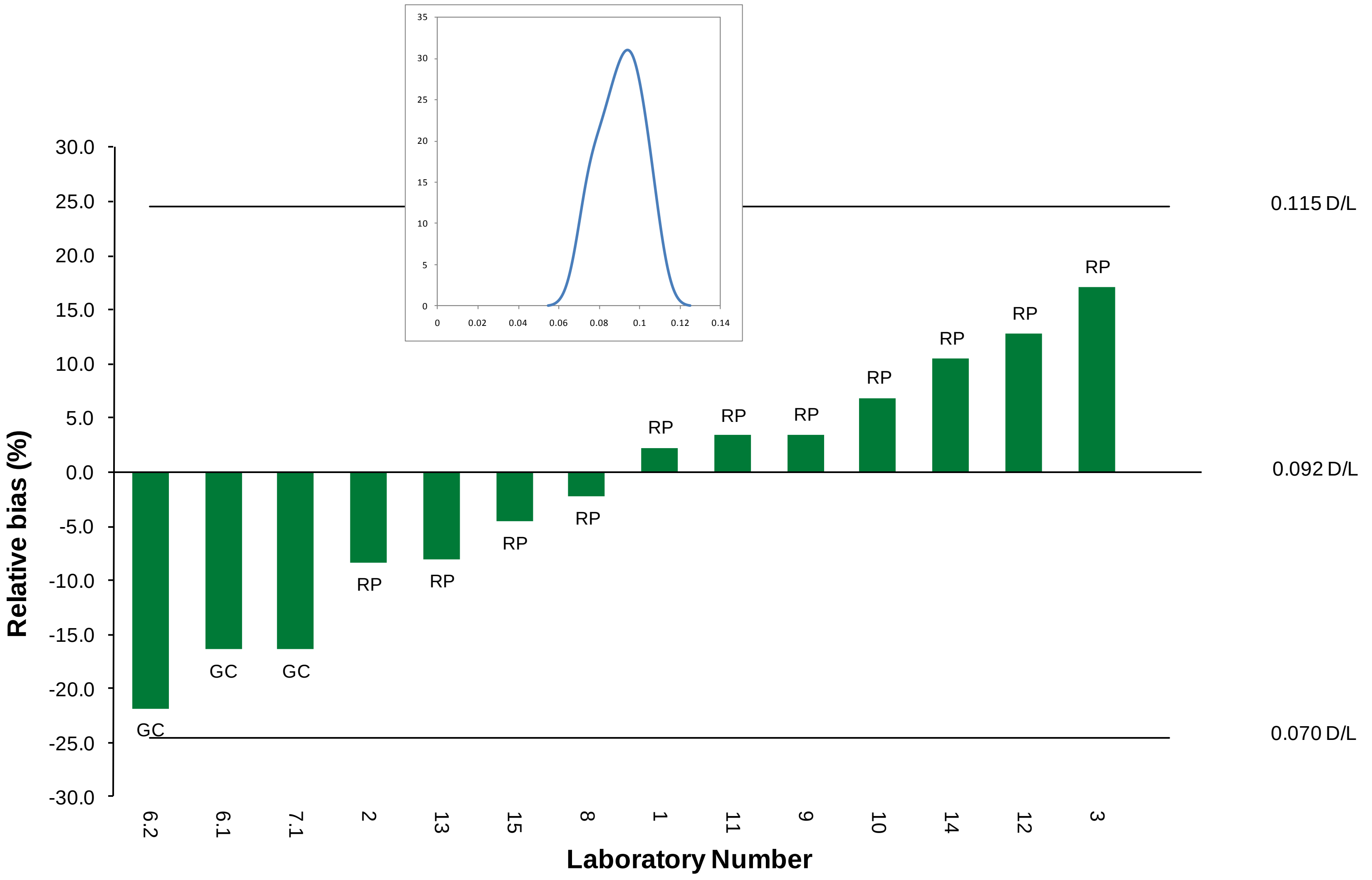


Figure 5.9: Relative Percentage Bias for **Alanine D/L Results (rpHPLC data only)** in Ostrich Egg Shell (A) Test Material

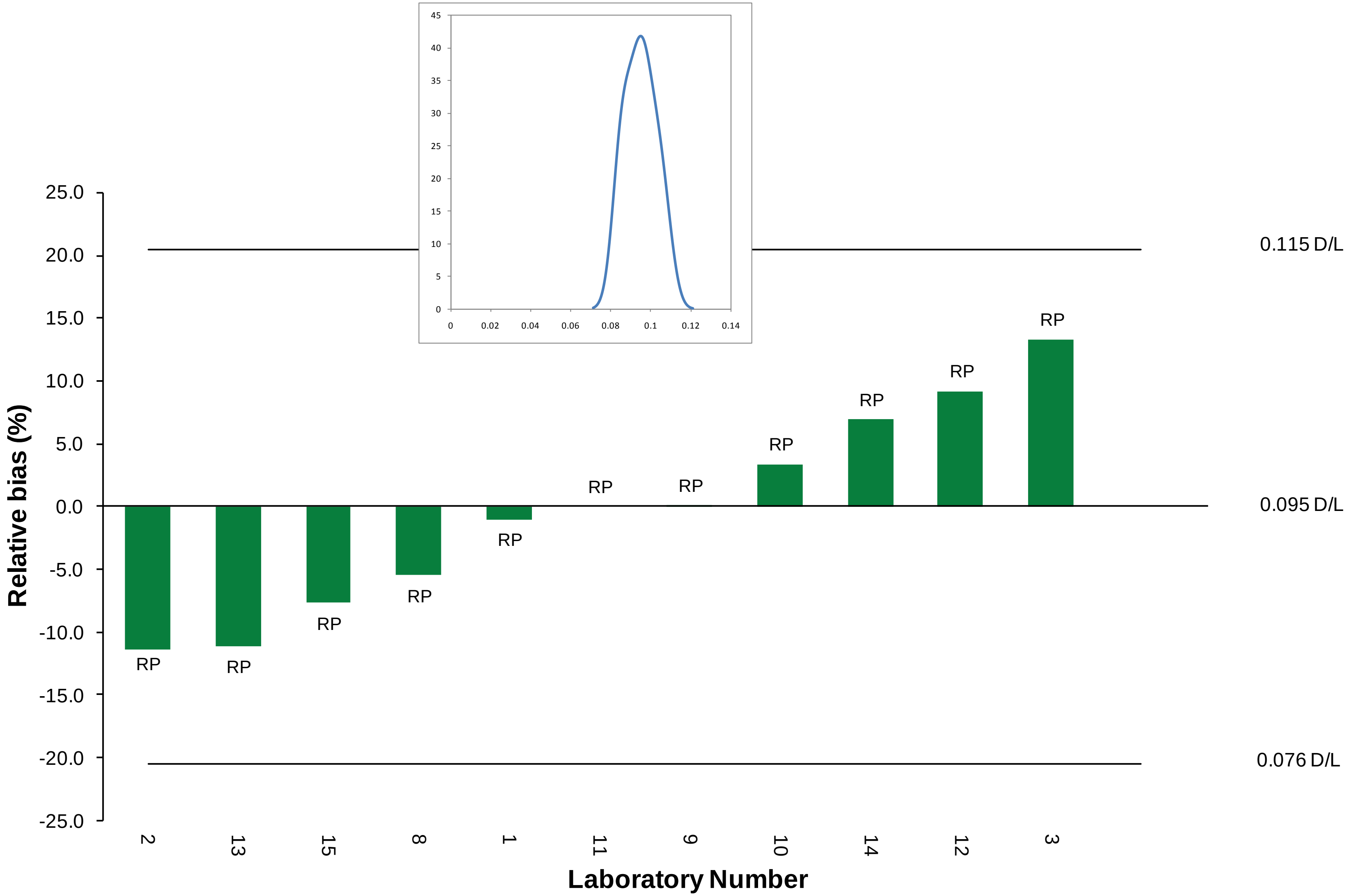


Figure 5.10: Relative Percentage Bias for **Valine D/L Results (all data)** in Ostrich Egg Shell (A) Test Material

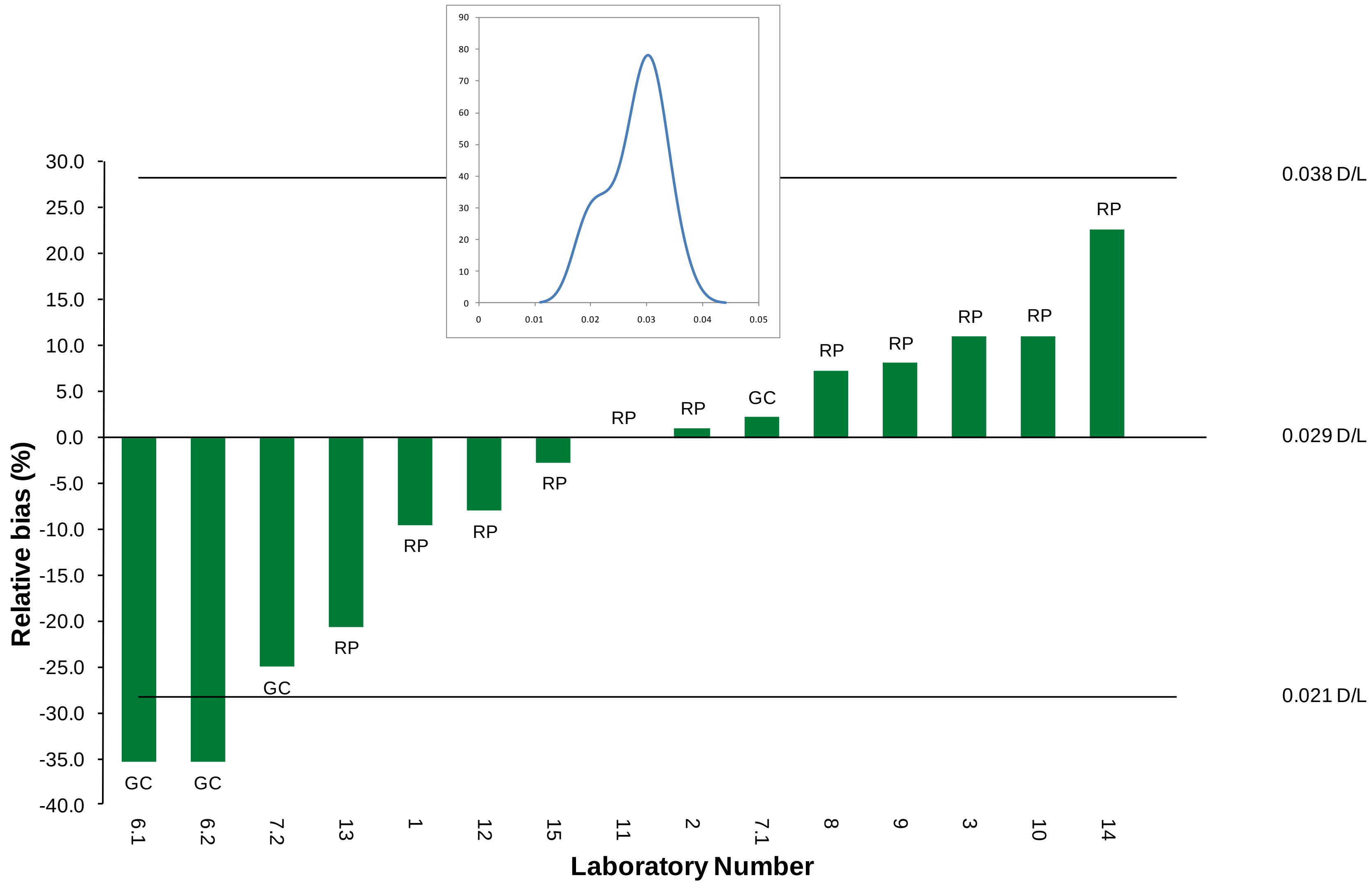


Figure 5.11: Relative Percentage Bias for **Valine D/L Results (rpHPLC data only)** in Ostrich Egg Shell (A) Test Material

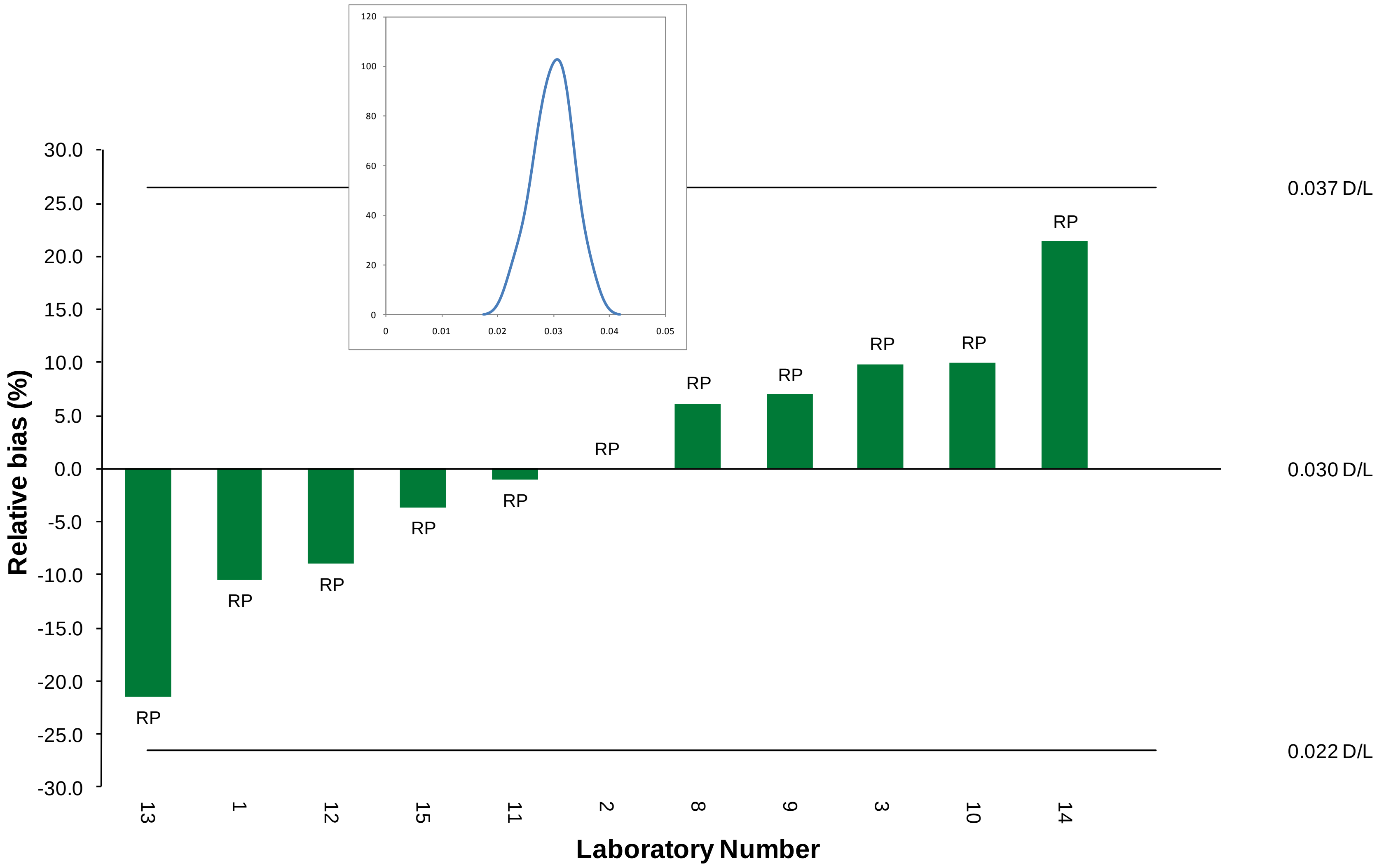


Figure 5.12: Relative Percentage Bias for **Phenylalanine D/L Results (all data)** in Ostrich Egg Shell (A) Test Material

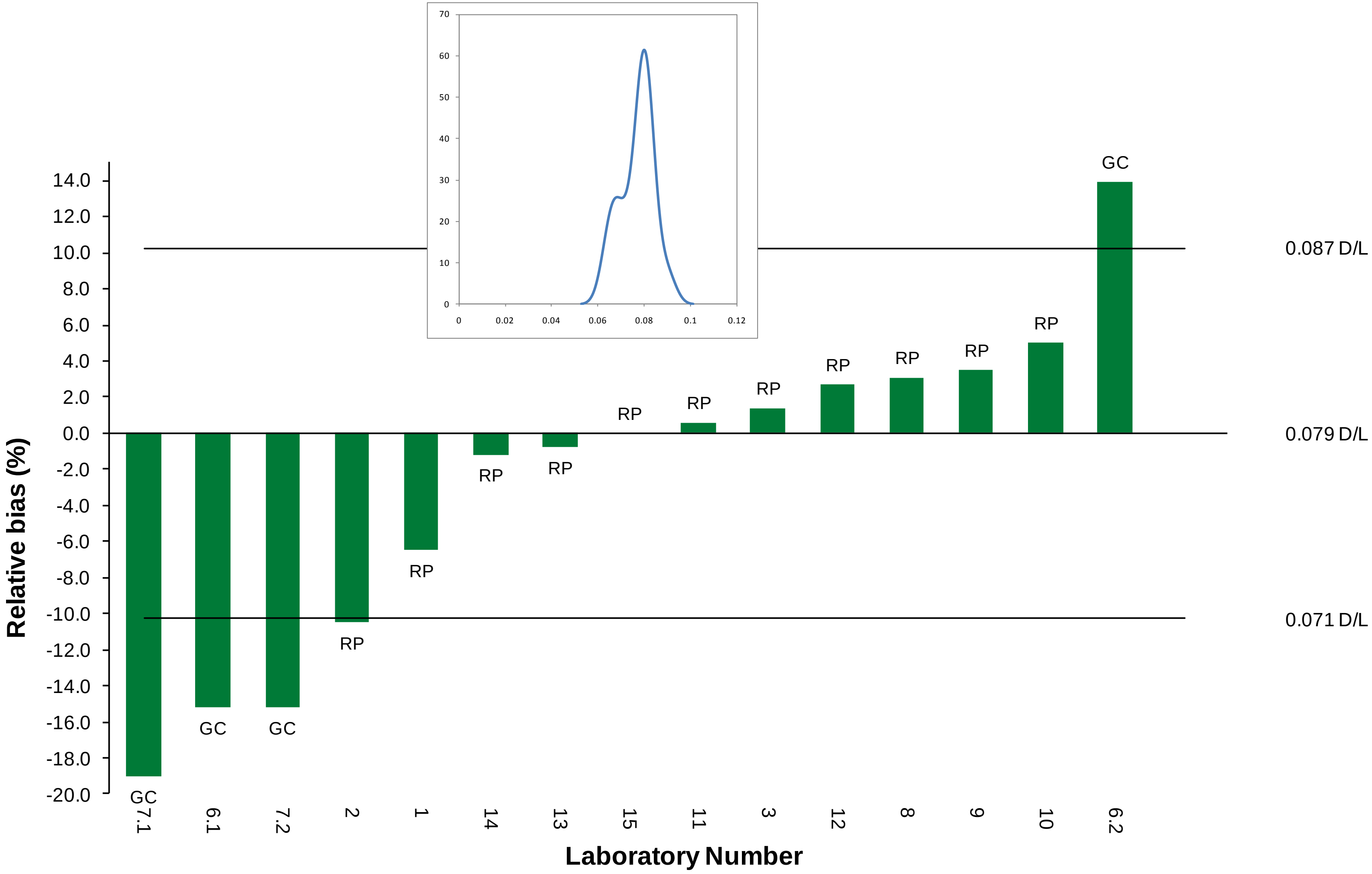


Figure 5.13: Relative Percentage Bias for **Phenylalanine D/L Results (rpHPLC data only)** in Ostrich Egg Shell (A) Test Material

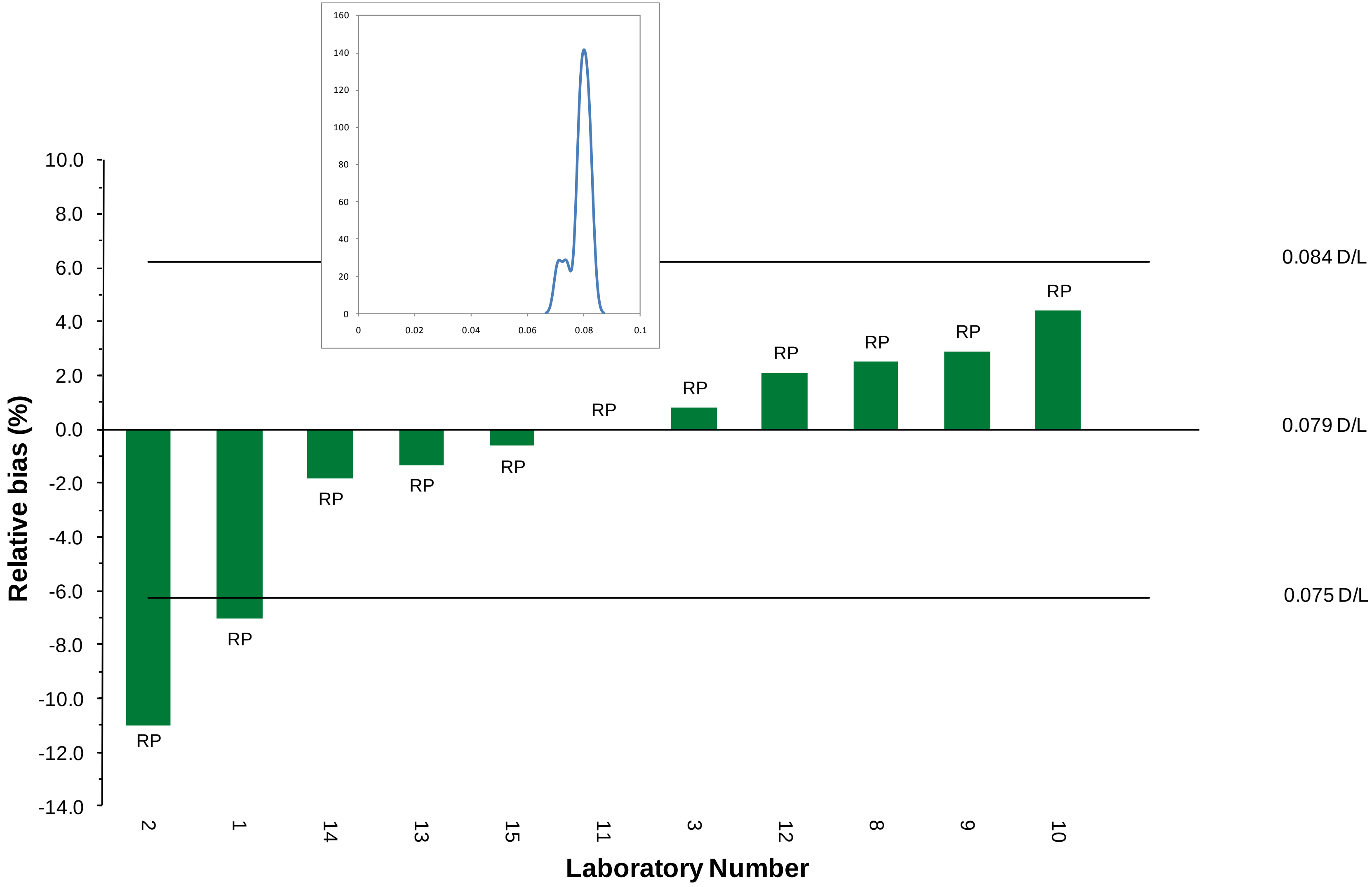


Figure 5.14: Relative Percentage Bias for **D-Alloisoleucine/L-Isoleucine Results (all data)** in Ostrich Egg Shell (A) Test Material

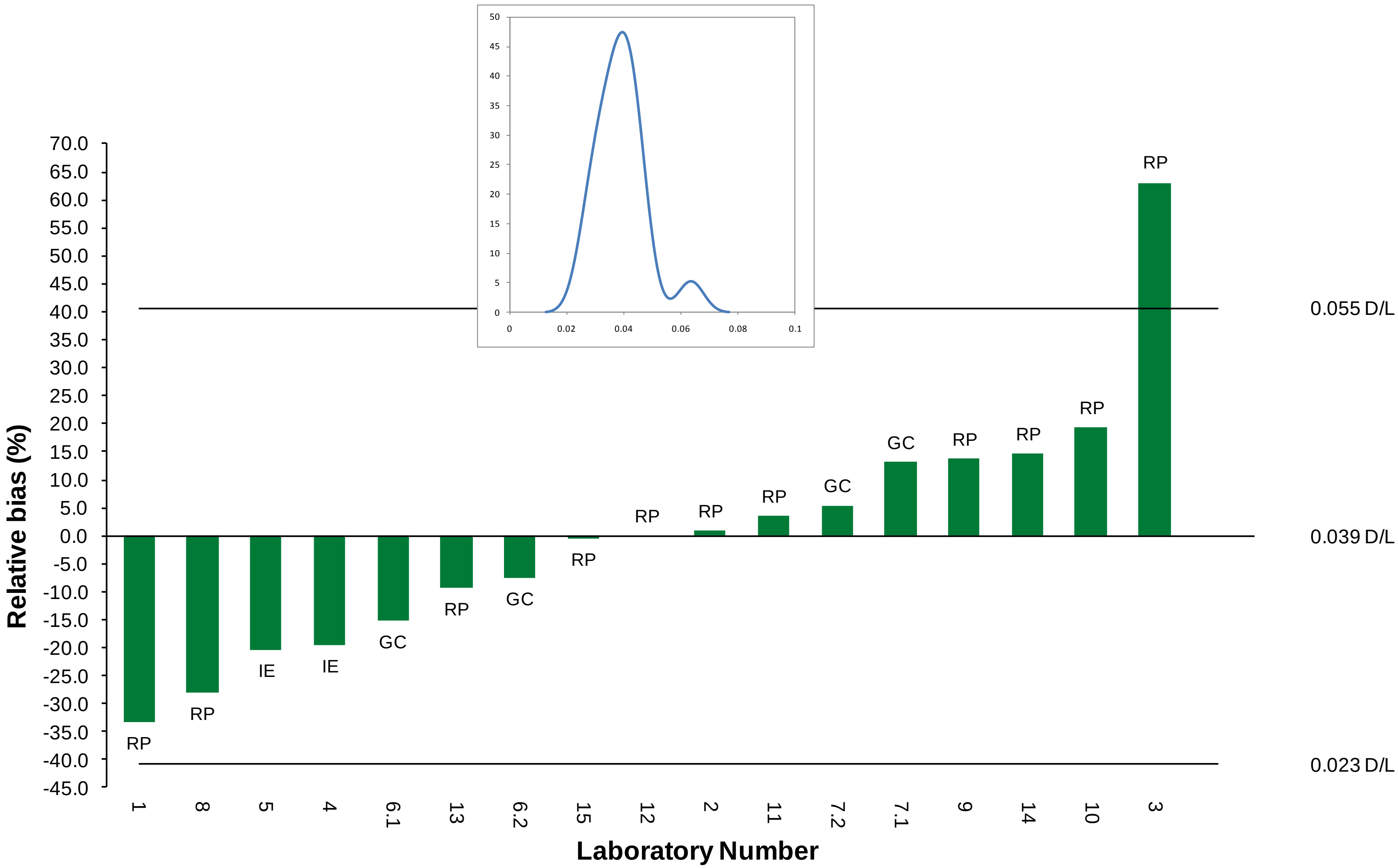


Figure 5.15: Relative Percentage Bias for **D-Alloisoleucine/L-Isoleucine Results (rpHPLC data only)** in Ostrich Egg Shell (A) Test Material

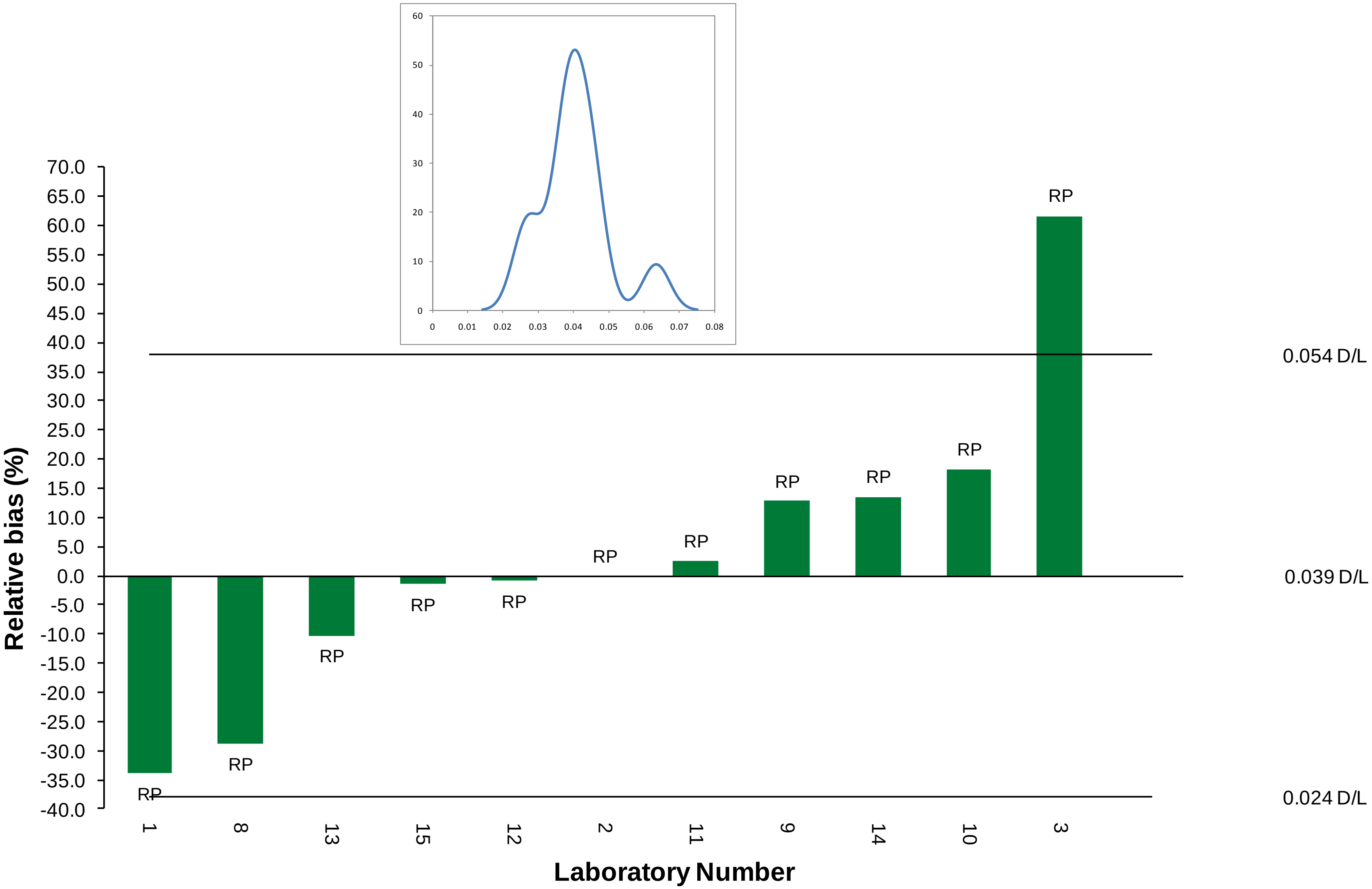


Figure 5.16: Relative Percentage Bias for **Leucine D/L Results (all data)** in Ostrich Egg Shell (A) Test Material

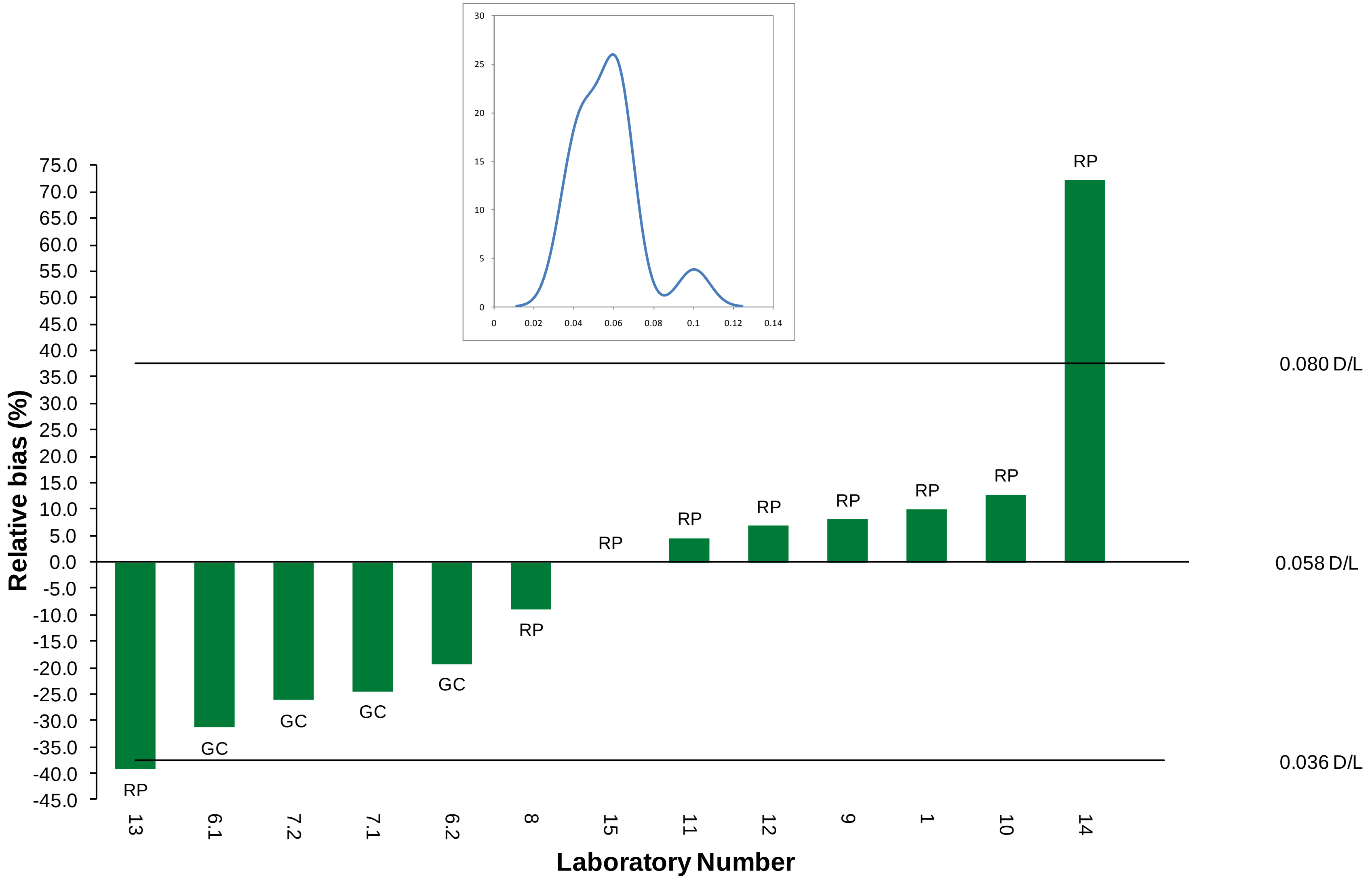


Figure 5.17: Relative Percentage Bias for **Leucine D/L Results (rpHPLC data only)** in Ostrich Egg Shell (A) Test Material

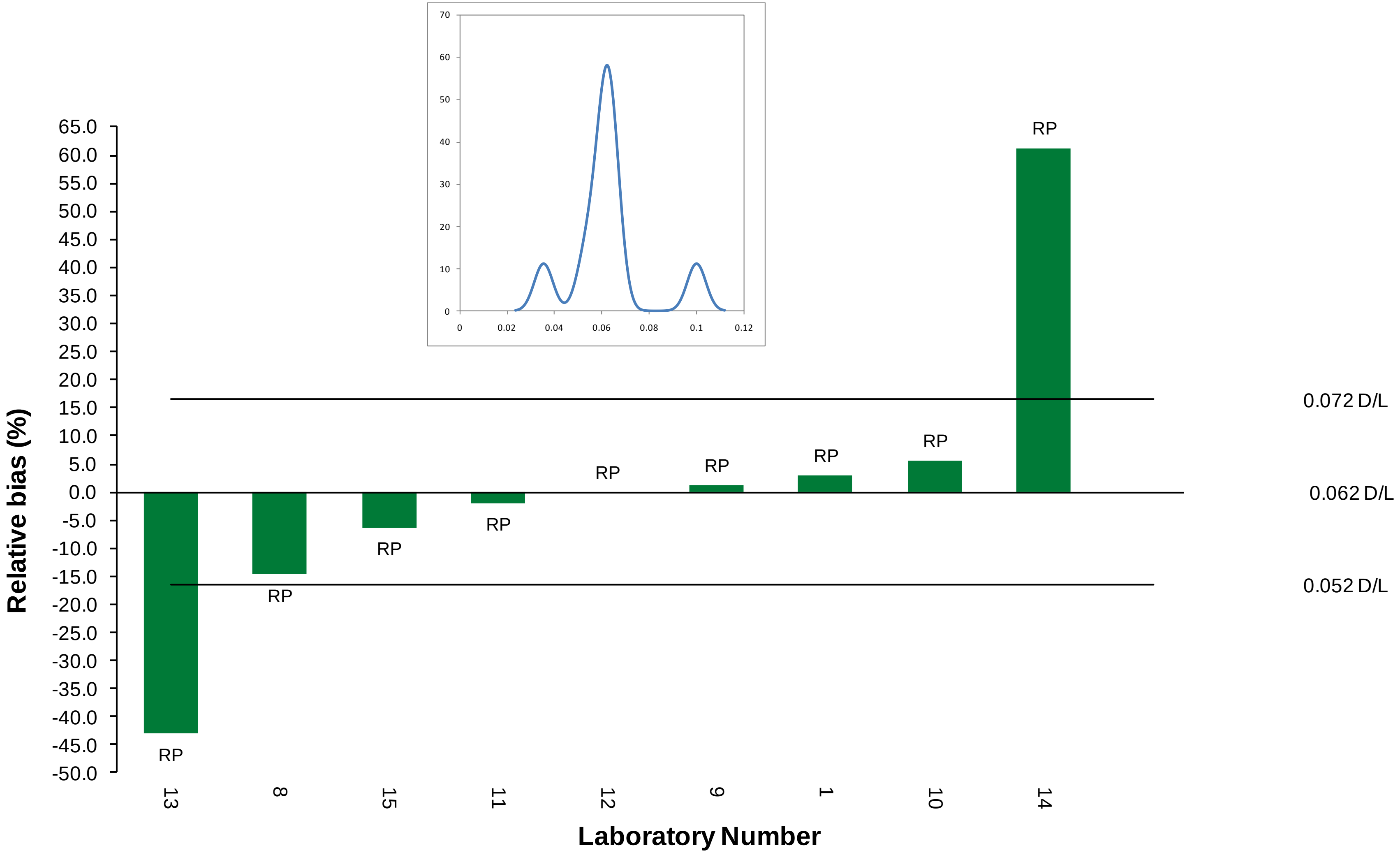
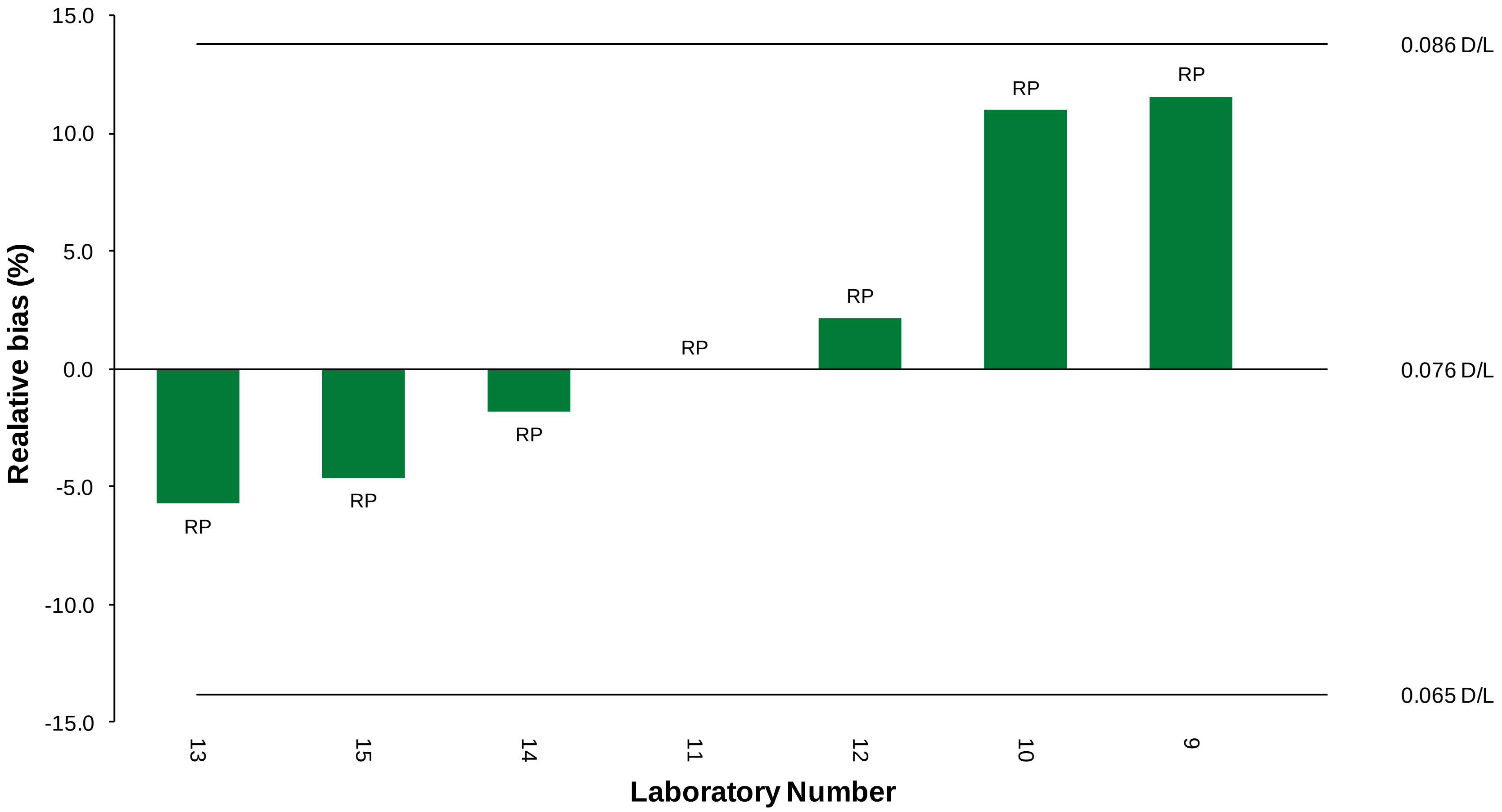


Figure 5.18: Relative Percentage Bias for **Tyrosine D/L Results (rpHPLC data only)** in Ostrich Egg Shell (A) Test Material



6 MEASUREMENT UNCERTAINTY

Ostrich Egg Shell (A) Test Material

6.1 Estimation of Measurement Uncertainty from Inter-laboratory comparisons.

Proficiency test data can provide a valuable indication of method and laboratory bias in routine analysis. Bias (*bias*) and its associated uncertainty (*u(bias)*)is often evaluated as part of a laboratory's method validation process by analysis of a certified reference material (CRM) or from spiking experiments. This, together with the determination of internal precision estimates (intra-laboratory reproducibility standard deviation (S_{Rw})) can define the overall combined uncertainty for a measurement system (u_C), and is referred to as the 'top-down' approach to measurement uncertainty determination (Barwick and Ellison, 2000).

Where such validation data is available, performance in a proficiency test can provide verification of a laboratory's own uncertainty estimates, which should be compatible with the spread of their PT results over time. However in the absence of such data the result can be used as a direct indication of bias itself, which together with an estimate of precision such as the intra-laboratory reproducibility standard deviation (S_{Rw}), can provide a value for the combined uncertainty.

It should be recognised that due to the uncertainty of the assigned value, bias and the uncertainty due to bias associated with a PT, The uncertainty estimate is likely to be larger than that resulting from the analysis of a CRM. It is recommended that long term bias trends are observed to lessen the impact from a single proficiency test result and at least 6 rounds of testing are used to evaluate bias estimates (Magnusson et al., 2004)

In addition, it is recommended that intra-laboratory precision estimates (S_{Rw}) are determined from replicate analyses of samples under reproducibility conditions over an extended period of time to take account of between run and general day to day variability. To simply use the standard deviation from replicate results submitted for the proficiency test is not a realistic representation of the overall method and laboratory precision. Alternatively, an estimation of the between laboratory reproducibility standard deviation (S_R) determined using an analysis of variance (ANOVA) on results from a collaborative trial, can be used directly in place of the combined standard uncertainty.

Thus; $$u_C = \sqrt{S_{Rw}{}^2 + u(bias)^2} \cong S_R$$

It is widely recognised that evaluation of PT data can be a valuable addition to the determination of measurement uncertainty, however there is very little information provided by the main guidance documents (JCGM 100:, 2008, EURACHEM / CITAC, 2000) on exactly how this should be done. The following methodology is therefore derived from two main sources; the Nordtest Report TR 537[iii] (Magnusson et al., 2004) produced as a handbook for the Nordic environmental testing laboratories and Eurolab's Technical reports[iv] Nos 1/2006 and 1/2007 (EUROLAB, 2006, EUROLAB, 2007). All documents are freely downloadable and recommended for further reading on the subject.

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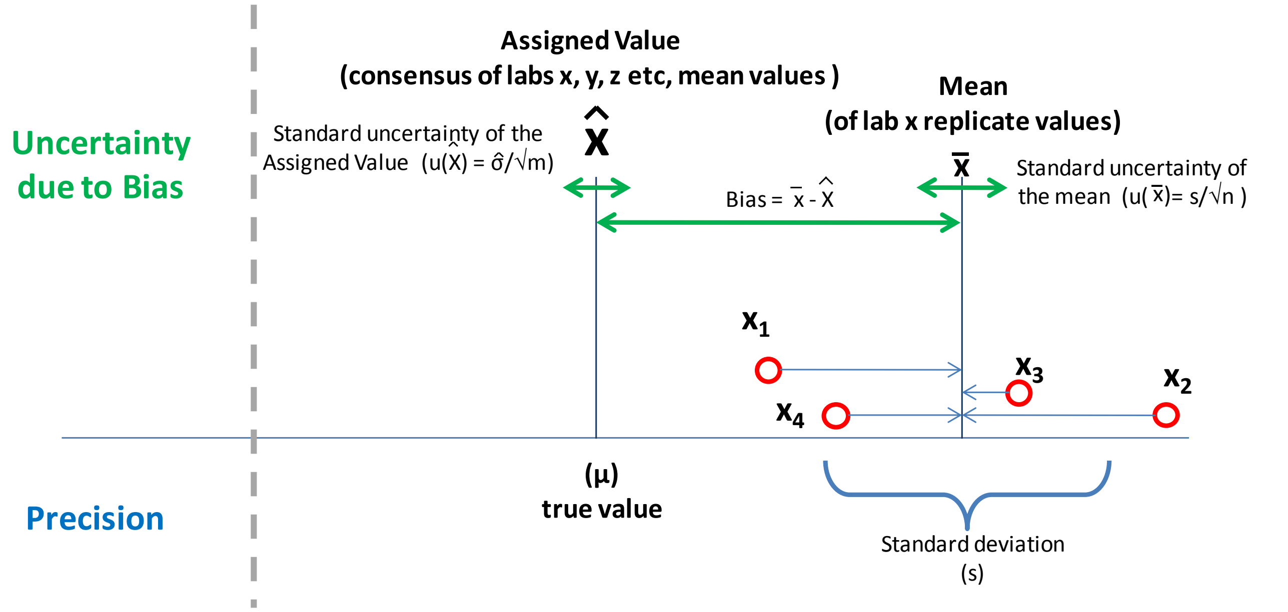
iii http://www.nordicinnovation.net/nordtestfiler/tec537.pdf
iv http://www.eurolab.org/pub/i_pub.html

For those readers unfamiliar with measurement uncertainty estimation, distinguishing the various uncertainty components can be somewhat baffling. Below helps to illustrate the sources and relevance of the different contributions due to precision and particularly those elements due to bias. These will now be expanded on in the remainder of this section, together with the calculation of the combined standard uncertainty and expanded uncertainty estimates.

Figure 6.1: Bias and Precision Components to Measurement Uncertainty Estimation.



6.2 Standard uncertainty due to Bias ($u(bias)$).

6.2.1 For a result from a single proficiency test.

The simplest expression for the bias uncertainty (*u(bias)*) is the experimental uncertainty of the laboratory mean $u(\bar{x})$ **plus** the uncertainty of the assigned value $u(\hat{X})$ where $u = s/\sqrt{n}$. Note; if a CRM was used as the test material, $u(\hat{X})$ can be taken from the specifications directly.

$$u(bias) = \sqrt{u(\bar{x})^2 + u(\hat{X})^2} = \sqrt{\frac{s_{\bar{x}}}{n_{\bar{x}}}^2 + \frac{s_{\hat{X}}}{m_{\hat{X}}}^2}$$

Where $s_{\bar{x}}$ = standard deviation of the laboratory's submitted result,
$n_{\bar{x}}$ = number of laboratory replicates,
$s_{\hat{X}}$ = standard deviation of the assigned value, and
$m_{\hat{X}}$ = number of laboratories' results contributing to the assigned value.

In routine analysis, bias should be accounted for and corrected for significant systematic effects. However in circumstances where this is not done by convention and the method is said to be empirical, any significant uncorrected bias should contribute to the combined uncertainty budget.

Bias is determined as ;

$bias = (\bar{x} - \hat{X})$ or as a relative value $\frac{bias}{\hat{X}} = \left(\frac{\bar{x}-\hat{X}}{\hat{X}}\right)$

Where $\bar{x}$ = laboratory result (or the mean of replicate values)
and $\hat{X}$ = the assigned value.

To determine whether the observed bias is significant or not, the *t* statistic is calculated and compared to the 2-tailed critical value for *n-1* degrees of freedom. If *t* is greater than or equal to the critical value, t_{crit} , then the bias is significant and an additional term to account for uncorrected bias in the result needs to be included in the combined uncertainty estimate (EURACHEM / CITAC, 2000).

t is calculated as;

$t = \frac{1-Rec}{u(Rec)}$ where ; $Rec = \bar{x}/\hat{X}$ and usually represents the recovery associated with the analysis of a CRM and $u(Rec)$ is the same as *u(bias)* given above.

If $t \geq t_{crit}$, *Rec* is significantly different from 1 and the result $\bar{x}$ remains uncorrected, a bias correction term needs to be included in the combined uncertainty estimate.

However, this scenario is to some extent academic as the uncertainty of the assigned value in a proficiency test is likely to be much larger than that of a CRM (if one were available) and it is recommended to include the bias contribution in the uncertainty evaluation at all times regardless of whether $t \geq t_{crit}$ or not (Magnusson et al., 2004).

Thus, the bias uncertainty now becomes;

$$u(bias) = \sqrt{(\bar{x} - \hat{X})^2 + \frac{S_{\bar{x}}}{n_{\bar{x}}}^2 + \frac{\hat{\sigma}}{m_{\hat{X}}}^2} \quad or \quad \sqrt{(bias)^2 + u(\bar{x})^2 + u(\hat{X})^2}$$

6.2.2 *For results from multiple proficiency tests*

When multiple results have been obtained from several proficiency tests then the contribution due to bias and the uncertainty due to bias (i.e.; the experimental uncertainty of the replicate mean $u(\bar{x})$), can be replaced by the bias root mean square (RMS_{bias}), thus;

$$u(bias) = \sqrt{RMS_{bias}^{\ 2} + u(\hat{X})^2} \quad \text{where} \quad RMS_{bias} = \sqrt{\sum (bias_i)^2 / m}$$

The average standard deviation for the assigned values and the average number of participants across all the tests can be determined and used to calculate an average uncertainty value for the tests.

"The use of an RMS value is equivalent to an estimated standard deviation around an assumed value of bias equal to zero. This implies that the RMS value takes into account both the bias and the variation of bias". (EUROLAB, 2007).

6.3 Combined uncertainty (u_C).

The combined uncertainty is therefore calculated as;

$$u_C = \sqrt{S_{Rw}^2 + u(\bar{x})^2 + u(\hat{X})^2 + (bias)^2}$$

Where S_{Rw} is the intra-laboratory reproducibility precision estimate.

Note concerning z-scores; for laboratories performing within the satisfactory range, i.e.; |z|=2, where there is a normal distribution of z-scores , that is, some may be positive and others negative, there will be no overall bias associated with the laboratory's performance. In this case the uncertainty associated with a result will be based on the uncertainty of that result, i.e.; $u(\bar{x})$, plus the uncertainty of the assigned value $u(\hat{X})$, plus the precision contributionS_{Rw}, which in this case is equivalent to the target standard deviation, σ_p. Where the uncertainty of the assigned value and /or the uncertainty of the result is considered negligible compared to the target standard deviation used for assessment (σ_p), then the uncertainty associated with the laboratory's result is simply equivalent to σ_p, or it's RSD value expressed as a percentage.

6.4 Expanded Uncertainty (U).

The final step in determining the measurement uncertainty is to calculate the Expanded uncertainty U by multiplying the combined uncertainty with a coverage factor *k*.

$U = u_c \times k$ where k is the coverage factor set according to the required confidence level.

For a discussion of the appropriate value of k, see Section 4.2.2. However, for a large, normally distributed data set, at a 95% or 2 standard deviation confidence level, k=2. For smaller data sets k=$t_{(0.05,df)}$.

A combined uncertainty brings together uncertainty contributions from different sources, therefore determining *k* becomes a little more tricky as there is no single value for the degrees of freedom. One approach is to calculate an effective degree of freedom using the Welch-Satterthwaite formula where the effective degree of freedom is less than or equal to the sum of the individual values, i.e.; $(v_{eff} \leq \sum v_i)$. The use of this equation is covered in detail in Annex G of the Guide to Uncertainty Measurement or "GUM"; (JCGM 100:, 2008).

$$v_{eff} = u_c^4(y) / \sum \frac{u_i^4(y)}{v_i}$$

Where	v_{eff}	=	the effective degrees of freedom,
	v_i	=	degrees of freedom of individual uncertainty components,
	u_c	=	combined standard uncertainty
	u_i	=	individual uncertainty components.

However, Eurachem make the following recommendation; "*Where the combined standard uncertainty is dominated by a single contribution with fewer than six degrees of freedom, it is recommended that k be set equal to the two-tailed value of the Student's t for the number of degrees of freedom associated with that contribution and for the level of confidence required…*" (EURACHEM / CITAC, 2000).

6.5 Calculating Measurement Uncertainty for Amino Acids in Ostrich Egg Shell (A) Test Material

To illustrate how precision and bias components can be used to provide an estimate of analytical uncertainty, the following evaluations have been carried. The information thus presented should perhaps be considered more as an information exercise than a definitive measure of uncertainty. This is due to a number of reasons; such as the relatively small data set, the "uncertainty" surrounding the empirical nature of the results and the effect on the confidence in the assigned value. Also because of the absence of true intra-laboratory precision estimates and the fact that not all laboratories supplied analytical replicate values. Nonetheless, the data presented in the following tables demonstrates how it can be possible to determine measurement uncertainty using proficiency test data and provides some interesting indicative values.

In all cases, individual laboratory expanded uncertainties (U) have been determined using a coverage factor k=2. This is to simplify the calculations whilst considering uncertainty components from various sources but also in order to enable direct comparability between laboratories and across analytes.

Results should be expressed as; result ($\bar{x}$) ± U (at 95% confidence, using k=2)

6.5.1 Measurement Uncertainty Evaluation for a series of results using RMS_{bias}.

As already mentioned in Section 6.3, for PT results with no overall bias ($bias$), where the uncertainty of the assigned values, $u(\hat{X})$, were negligible and where the uncertainty of replicate values, $u(\bar{x})$ were small compared to intra-laboratory precision estimates S_{Rw}, then the standard uncertainty for laboratories within the satisfactory range would be equivalent to the target standard deviation, σ_p.

However, in this report, no values for target standard deviation, σ_p, have been given. Under these circumstances and assuming the absence of bias described above still holds, the uncertainty of laboratories' mean values would be equivalent to each laboratory's own intra-laboratory reproducibility S_{Rw}, if this information where known. In the absence of this, the instrumental repeatability (i.e.; the RSD% or CV%) derived from the replicate values might be used, ideally with an additional term included to take into account the expected variability between samples. In the absence of this and to avoid the risk of under-valuing the precision contribution, the reproducibility value derived from all participant's results, given in Table 4.1 at the beginning of the report, might be used as a compromise. This would assume that all laboratories were performing at the stated level of precision and makes no allowance for those that were performing better or worse than this.

Whilst the above scenario may be ideal, in reality it is probably a little unrealistic. It would be far more appropriate to assess the bias components and include them in the uncertainty budget, even if their overall contribution is small, at least until the analyst is confident that analytical results are free from bias.

Table 6.1 demonstrates how this could be carried out using a series of results. In this example we are using results from a number of laboratories in a single round of testing to obtain an average uncertainty for the amino acid in the test material. In practice it is perhaps more likely that a single laboratory would want to assess their own data from a series of proficiency tests carried out. The data shown uses the RMS_{bias}% (see 6.2.2) determined from all the submitted results by all the laboratories for any given amino acid. From this the average combined and expanded uncertainties for each amino acid for this test material can be derived.

Here the precision estimates used are the standard deviations for the assigned values, ($\hat{\sigma}$), i.e.; sMAD (see Section 5.3). They represent the distributions of the laboratories' means and were used to set the satisfactory limits (i.e.; ± 2 std dev),.but are not as influenced as the reproducibility standard deviations (S_R and RSD_R%) given in Table 4.1, by poor repeatability of the replicate results and extreme values. (Although in practice each laboratory should use their own intra-laboratory reproducibility (S_{RW}) precision estimate for the analyte in question and the different laboratories would be replaced by results from different rounds of testing for any given laboratory). Nonetheless, the average uncertainty for each amino acid calculated across all the laboratories still provides some interesting results which can be compared to the individual values calculated next.

6.5.2 Measurement Uncertainty Evaluation for a single result.

Table 6.2 then looks at individual laboratory uncertainty estimates for each amino acid. Although this approach is not recommended and long term trends (as described above), give more appropriate approximations, it can be helpful to observe unexpected random error effects between rounds of proficiency testing. Here the individual bias components have been assessed separately as discussed in Section 6.2.1 and the CV% or RSD% determined from instrumental replicates have been used where available, in place the laboratory's own estimation of precision for that analyte, S_{RW}. However it should be noted that precision based on instrument repeatability is likely to be small compared to any long term true intra-laboratory reproducibility (intermediate precision) estimate and may contribute to smaller expanded uncertainties than might be otherwise expected.

Individual laboratory standard uncertainty components have been presented as histograms, together with each laboratory's combined uncertainty value and the average combined uncertainty for the test material described in the previous section and given in Table 6.1. In addition, expanded uncertainty confidence intervals have been determined and plotted for each amino acid to illustrate the effect of uncertainty on the mean of submitted results.

Table 6.1: Estimation of Relative Standard Uncertainty, Combined and Expanded Uncertainty for Amino Acids (using RMS_{bias}% to access bias contributions) across ALL Laboratories.

analyte	Std uncertainty contributions			Combined & Expanded uncertainties	
	Precision[1]	Bias components[2,3]			
	1	2	3		
	$\hat{\sigma}$ as RSD%	$u(\hat{X})$as RSU%	RMS_{bias}%	combined u_c%	Expanded $U\%\ (k=2)$
Asx D/L (all[a])	3.84	0.99	6.96	8.01	16.02
Asx D/L (rpHPLC)	3.76	1.13	3.14	5.03	10.06
Glx D/L (all[a])	8.32	2.15	9.69	12.95	25.90
Glx D/L (rpHPLC)	12.72	3.83	9.45	16.30	32.61
Ser D/L (rpHPLC)	1.27	0.38	3.35	3.60	7.20
Arg D/L (rpHPLC)	11.56	3.85	15.60	19.79	39.58
Ala D/L (all[a])	12.25	3.27	10.99	16.78	33.56
Ala D/L (rpHPLC)	10.25	3.09	7.12	12.85	25.71
Val D/L (all[a])	14.13	3.65	16.46	22.00	43.99
Val D/L (rpHPLC)	13.23	3.99	9.22	16.61	33.22
Phe D/L (all[a])	5.14	1.33	9.05	10.49	20.98
Phe D/L (rpHPLC)	3.12	0.94	4.38	5.46	10.92
D-Aile/L-Ile (all[b])	20.37	4.94	21.45	29.99	59.98
D-Aile/L-Ile (rpHPLC)	18.92	5.71	24.10	31.17	62.33
Leu D/L (all[a])	18.81	5.22	18.87	27.15	54.30
Leu D/L (rpHPLC)	8.24	2.75	15.32	17.61	35.22
Tyr D/L (rpHPLC)	6.89	2.61	6.48	9.81	19.63

Notes for Table 6.1:

[a] = rpHPLC and GC data [b] = rpHPLC, GC and HPLC-IE data

[1] = $\hat{\sigma}$ is the standard deviation for the assigned value, i.e., the median absolute deviation (sMAD), expressed as a percentage (given in Table 5.2).

[2] =$u(\hat{X})$ is the uncertainty of the assigned value $(\hat{X})$ expressed as a percentage, (given in Table 5.2).

[3] = RMS_{bias} is the observed uncertainty due to bias of the submitted results

Table 6.2: Estimation of Relative Standard Uncertainty, Combined and Expanded Uncertainty Estimations for Individual Laboratories

laboratory number	mean result	Std uncertainty contributions: Precision[4]	Bias components[5,6,7]			Combined & Expanded uncertainties	
Asx D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%$ $(k=2)$
1	0.350	1.44	0.99	0.48	7.62	7.84	15.67
2	0.349	0.37	0.99	0.26	7.82	7.90	15.79
3	0.365	n=1	0.99	n=1	3.62		
4							
5							
6.1	0.379	5.01	0.99	2.51	0.07	5.69	11.38
6.2	0.453	0.11	0.99	0.08	19.61	19.64	39.28
7.1	0.393	n=1	0.99	n=1	3.77		
7.2	0.427	n=1	0.99	n=1	12.75		
8	0.361	0.20	0.99	0.14	4.81	4.92	9.84
9	0.380	0.04	0.99	0.03	0.26	1.03	2.05
10	0.382	0.13	0.99	0.09	0.86	1.32	2.64
11	0.379	0.16	0.99	0.11	0.00	1.01	2.02
12	0.385	0.21	0.99	0.14	1.54	1.85	3.71
13	0.369	n=1	0.99	n=1	2.59		
14	0.377	n=1	0.99	n=1	0.36		
15	0.370	0.80	0.99	0.56	2.34	2.72	5.44
Asx D/L rpHPLC		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%$ $(k=2)$
1	0.350	1.44	1.13	0.48	5.42	5.74	11.47
2	0.349	0.37	1.13	0.26	5.62	5.75	11.49
3	0.365	n=1	1.13	n=1	1.32		
4							
5							
6.1							
6.2							
7.1							
7.2							
8	0.361	0.20	1.13	0.14	2.54	2.79	5.58
9	0.380	0.04	1.13	0.03	2.66	2.89	5.78
10	0.382	0.13	1.13	0.09	3.27	3.46	6.93
11	0.379	0.16	1.13	0.11	2.39	2.65	5.31
12	0.385	0.21	1.13	0.14	3.97	4.14	8.28
13	0.369	n=1	1.13	n=1	0.26		
14	0.377	n=1	1.13	n=1	2.03		
15	0.370	0.80	1.13	0.56	0.00	1.50	2.99

[4] = σ is the standard deviation of submitted results, expressed as a relative % i.e.; $CV\% = (\sigma/\bar{x}) \times 100$ (see Section 4).
[5] =$u(\hat{X})$ is the uncertainty of the assigned value $(\hat{X})$ expressed as a relative % i.e.; $RSU_{\hat{X}}\% = (u(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = $u(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\hat{x}}\% = (u(\bar{x})/\bar{x}) \times 100$ (see Section 4).
[7] = Relative bias expressed as a % i.e.; $Bias\% = (\bar{x} - \hat{X}/\hat{X}) \times 100$

Table 6.2: Estimation of Relative Standard Uncertainty, Combined and Expanded Uncertainty Estimations for (continued).

laboratory number	mean result	Std uncertainty contributions: Precision[4]	Bias components[5,6,7]			Combined & Expanded uncertainties	
Glx D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.078	7.38	2.15	2.61	9.90	12.80	25.60
2	0.069	0.46	2.15	0.32	20.00	20.12	40.25
3	0.075	n=1	2.15	n=1	13.19		
4							
5							
6.1	0.082	15.85	2.15	7.93	5.61	18.72	37.43
6.2	0.105	n=1	2.15	n=1	20.86		
7.1	0.086	n=1	2.15	n=1	1.01		
7.2	0.086	n=1	2.15	n=1	1.01		
8	0.085	0.00	2.15	0.00	2.16	3.05	6.09
9	0.095	0.30	2.15	0.21	9.22	9.47	18.95
10	0.094	0.24	2.15	0.17	8.43	8.71	17.41
11	0.092	0.51	2.15	0.36	5.54	5.97	11.95
12	0.094	0.15	2.15	0.11	8.74	9.00	18.00
13	0.087	n=1	2.15	n=1	0.00		
14	0.091	n=1	2.15	n=1	5.20		
15	0.088	0.66	2.15	0.47	1.86	2.95	5.90
Glx D/L rpHPLC		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.078	7.38	3.83	2.61	11.54	14.46	28.93
2	0.069	0.46	3.83	0.32	21.46	21.81	43.61
3	0.075	n=1	3.83	n=1	14.77		
4							
5							
6.1							
6.2							
7.1							
7.2							
8	0.085	0.00	3.83	0.00	3.94	5.50	11.00
9	0.095	0.30	3.83	0.21	7.23	8.19	16.38
10	0.094	0.24	3.83	0.17	6.45	7.51	15.03
11	0.092	0.51	3.83	0.36	3.61	5.31	10.61
12	0.094	0.15	3.83	0.11	6.76	7.77	15.54
13	0.087	n=1	3.83	n=1	1.82		
14	0.091	n=1	3.83	n=1	3.29		
15	0.088	0.66	3.83	0.47	0.00	3.92	7.84

[4] = σ is the standard deviation of submitted results, expressed as a relative % i.e.; $CV\% = (\sigma/\bar{x}) \times 100$ (see Section 4).
[5] =$u(\hat{X})$ is the uncertainty of the assigned value $(\hat{X})$ expressed as a relative % i.e.; $RSU_{\hat{X}}\% = (u(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = $u(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\hat{x}}\% = (u(\bar{x})/\bar{x}) \times 100$ (see Section 4).
[7] = Relative bias expressed as a % i.e.; $Bias\% = (\bar{x} - \hat{X}/\hat{X}) \times 100$

Table 6.2: Estimation of Relative Standard Uncertainty, Combined and Expanded Uncertainty Estimations for Individual Laboratories (continued).

laboratory number	mean result	Std uncertainty contributions: Precision[4]	Bias components[5,6,7]			Combined & Expanded uncertainties	
Ser D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.329	0.62	0.38	0.20	0.64	0.99	1.98
2	0.326	1.99	0.38	1.41	0.29	2.48	4.97
3	0.356	n=1	0.38	n=1	9.05		
4							
5							
6.1							
6.2							
7.1							
7.2							
8	0.345	0.41	0.38	0.29	5.68	5.71	11.42
9	0.326	0.44	0.38	0.31	0.19	0.68	1.36
10	0.332	0.14	0.38	0.10	1.77	1.82	3.63
11	0.325	0.17	0.38	0.12	0.49	0.65	1.31
12	0.329	0.00	0.38	0.00	0.85	0.94	1.87
13	0.319	n=1	0.38	n=1	2.18		
14	0.326	n=1	0.38	n=1	0.00		
15	0.318	0.44	0.38	0.31	2.55	2.63	5.26
Arg D/L rpHPLC		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1							
2	0.125	0.50	3.85	0.35	6.58	7.65	15.30
3	0.184	n=1	3.85	n=1	37.28		
4							
5							
6.1							
6.2							
7.1							
7.2							
8							
9	0.160	5.54	3.85	3.92	19.48	20.98	41.97
10	0.153	3.63	3.85	2.56	14.17	15.34	30.68
11	0.126	0.84	3.85	0.59	5.95	7.16	14.32
12	0.122	0.55	3.85	0.39	8.93	9.75	19.50
13	0.124	n=1	3.85	n=1	7.79		
14	0.134	n=1	3.85	n=1	0.00		
15	0.135	6.21	3.85	4.39	0.44	8.53	17.07

[4] = σ is the standard deviation of submitted results, expressed as a relative % i.e.; $CV\% = (\sigma/\bar{x}) \times 100$ (see Section 4).
[5] =$u(\hat{X})$ is the uncertainty of the assigned value $(\hat{X})$ expressed as a relative % i.e.; $RSU_{\hat{X}}\% = (u(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = $u(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\bar{x}}\% = (u(\bar{x})/\bar{x}) \times 100$ (see Section 4).
[7] = Relative bias expressed as a % i.e.; $Bias\% = (\bar{x} - \hat{X}/\hat{X}) \times 100$

Table 6.2: Estimation of Relative Standard Uncertainty, Combined and Expanded Uncertainty Estimations for Individual Laboratories (continued).

laboratory number	mean result	Std uncertainty contributions Precision[4]	Bias components[5,6,7]			Combined & Expanded uncertainties	
Ala D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.094	3.95	3.27	1.25	2.30	5.76	11.51
2	0.084	0.29	3.27	0.21	8.43	9.05	18.09
3	0.108	n=1	3.27	n=1	17.14		
4							
5							
6.1	0.077	5.19	3.27	1.96	16.41	17.63	35.26
6.2	0.072	1.39	3.27	0.62	21.84	22.13	44.27
7.1	0.077	n=1	3.27	n=1	16.41		
7.2							
8	0.090	0.00	3.27	0.00	2.30	4.00	8.00
9	0.095	0.63	3.27	0.45	3.46	4.83	9.66
10	0.098	1.52	3.27	1.07	6.89	7.85	15.70
11	0.095	4.70	3.27	3.32	3.36	7.42	14.85
12	0.104	0.03	3.27	0.02	12.85	13.26	26.52
13	0.085	n=1	3.27	n=1	8.10		
14	0.102	n=1	3.27	n=1	10.50		
15	0.088	3.05	3.27	2.16	4.65	6.81	13.61
Ala D/L (rpHPLC)		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.094	3.95	3.09	1.25	1.03	5.27	10.53
2	0.084	0.29	3.09	0.21	11.40	11.82	23.63
3	0.108	n=1	3.09	n=1	13.34		
4							
5							
6.1							
6.2							
7.1							
7.2							
8	0.090	0.00	3.09	0.00	5.47	6.28	12.56
9	0.095	0.63	3.09	0.45	0.10	3.19	6.37
10	0.098	1.52	3.09	1.07	3.42	4.97	9.93
11	0.095	4.70	3.09	3.32	0.00	6.53	13.06
12	0.104	0.03	3.09	0.02	9.18	9.69	19.38
13	0.085	n=1	3.09	n=1	11.09		
14	0.102	n=1	3.09	n=1	6.91		
15	0.088	3.05	3.09	2.16	7.75	9.14	18.28

[4] = σ is the standard deviation of submitted results, expressed as a relative % i.e.; $CV\% = (\sigma/\bar{x}) \times 100$ (see Section 4).
[5] =$u(\hat{X})$ is the uncertainty of the assigned value $(\hat{X})$ expressed as a relative % i.e.; $RSU_{\hat{X}}\% = (u(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = $u(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\bar{x}}\% = (u(\bar{x})/\bar{x}) \times 100$ (see Section 4).
[7] = Relative bias expressed as a % i.e.; $Bias\% = (\bar{x} - \hat{X}/\hat{X}) \times 100$

Table 6.2: Estimation of Relative Standard Uncertainty, Combined and Expanded Uncertainty Estimations for Individual Laboratories (continued).

laboratory number	mean result	Std uncertainty contributions Precision[4]	Bias components[5,6,7]			Combined & Expanded uncertainties	
Val D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.027	10.64	3.65	3.37	9.53	15.12	30.25
2	0.030	1.33	3.65	0.94	1.03	4.13	8.25
3	0.033	n=1	3.65	n=1	11.03		
4							
5							
6.1	0.019	15.79	3.65	6.45	35.27	39.34	78.68
6.2	0.019	5.26	3.65	3.04	35.27	35.97	71.94
7.1	0.030	n=1	3.65	n=1	2.21		
7.2	0.022	n=1	3.65	n=1	25.04		
8	0.032	2.24	3.65	1.59	7.32	8.63	17.26
9	0.032	4.52	3.65	3.20	8.11	10.47	20.95
10	0.033	1.95	3.65	1.38	11.10	11.92	23.85
11	0.029	2.55	3.65	1.81	0.00	4.80	9.61
12	0.027	1.02	3.65	0.72	7.99	8.87	17.74
13	0.023	n=1	3.65	n=1	20.63		
14	0.036	n=1	3.65	n=1	22.70		
15	0.029	4.15	3.65	2.94	2.66	6.80	13.60
Val D/L (rpHPLC)		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.027	10.64	3.99	3.37	10.45	15.80	31.61
2	0.030	1.33	3.99	0.94	0.00	4.31	8.62
3	0.033	n=1	3.99	n=1	9.90		
4							
5							
6.1							
6.2							
7.1							
7.2							
8	0.032	2.24	3.99	1.59	6.23	7.89	15.79
9	0.032	4.52	3.99	3.20	7.01	9.78	19.56
10	0.033	1.95	3.99	1.38	9.97	11.00	22.00
11	0.029	2.55	3.99	1.81	1.02	5.17	10.34
12	0.027	1.02	3.99	0.72	8.92	9.85	19.71
13	0.023	n=1	3.99	n=1	21.43		
14	0.036	n=1	3.99	n=1	21.45		
15	0.029	4.15	3.99	2.94	3.65	7.42	14.84

4 = σ is the standard deviation of submitted results, expressed as a relative % i.e.; $CV\% = (\sigma/\bar{x}) \times 100$ (see Section 4).
5 =$u(\hat{X})$ is the uncertainty of the assigned value $(\hat{X})$ expressed as a relative % i.e.; $RSU_{\hat{X}}\% = (u(\hat{X})/\hat{X}) \times 100$ (see Section 5)
6 = $u(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\bar{x}}\% = (u(\bar{x})/\bar{x}) \times 100$ (see Section 4).
7 = Relative bias expressed as a % i.e.; $Bias\% = (\bar{x} - \hat{X}/\hat{X}) \times 100$

Table 6.2: Estimation of Relative Standard Uncertainty, Combined and Expanded Uncertainty Estimations for Individual Laboratories (continued).

laboratory number	mean result	Std uncertainty contributions: Precision[4]	Bias components[5,6,7]			Combined & Expanded uncertainties	
Phe D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.074	4.41	1.33	1.39	6.52	8.10	16.20
2	0.071	0.30	1.33	0.21	10.47	10.56	21.13
3	0.080	n=1	1.33	n=1	1.41		
4							
5							
6.1	0.067	11.94	1.33	4.51	15.24	19.92	39.84
6.2	0.090	n=1	1.33	n=1	13.86		
7.1	0.064	n=1	1.33	n=1	19.03		
7.2	0.067	n=1	1.33	n=1	15.24		
8	0.082	11.28	1.33	7.98	3.11	14.22	28.44
9	0.082	0.14	1.33	0.10	3.46	3.71	7.43
10	0.083	0.79	1.33	0.56	4.96	5.22	10.45
11	0.079	0.80	1.33	0.57	0.56	1.74	3.49
12	0.081	0.29	1.33	0.21	2.68	3.01	6.02
13	0.078	n=1	1.33	n=1	0.76		
14	0.078	n=1	1.33	n=1	1.20		
15	0.079	2.77	1.33	1.96	0.00	3.65	7.30
Phe D/L (rpHPLC)		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.074	4.41	0.94	1.39	7.04	8.48	16.96
2	0.071	0.30	0.94	0.21	10.98	11.02	22.04
3	0.080	n=1	0.94	n=1	0.84		
4							
5							
6.1							
6.2							
7.1							
7.2							
8	0.082	11.28	0.94	7.98	2.53	14.07	28.15
9	0.082	0.14	0.94	0.10	2.89	3.04	6.08
10	0.083	0.79	0.94	0.56	4.37	4.57	9.15
11	0.079	0.80	0.94	0.57	0.00	1.36	2.72
12	0.081	0.29	0.94	0.21	2.11	2.33	4.67
13	0.078	n=1	0.94	n=1	1.31		
14	0.078	n=1	0.94	n=1	1.75		
15	0.079	2.77	0.94	1.96	0.56	3.57	7.14

[4] = σ is the standard deviation of submitted results, expressed as a relative % i.e.; $CV\% = (\sigma/\bar{x}) \times 100$ (see Section 4).
[5] =u(X̂) is the uncertainty of the assigned value (X̂) expressed as a relative % i.e.; $RSU_{\hat{X}}\% = (u(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = u(x̄) is the bias standard deviation for submitted results (x̄) expressed as a relative % $RSU_{\hat{x}}\% = (u(\bar{x})/\bar{x}) \times 100$ (see Section 4).
[7] = Relative bias expressed as a % i.e.; $Bias\% = (\bar{x} - \hat{X}/\hat{X}) \times 100$

Table 6.2: Estimation of Relative Standard Uncertainty, Combined and Expanded Uncertainty Estimations for Individual Laboratories (continued).

laboratory number	mean result	Std uncertainty contributions: Precision[4]	Bias components[5,6,7]			Combined & Expanded uncertainties	
D-Aile/L-Ile		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.026	5.19	4.94	1.64	33.27	34.07	68.14
2	0.039	1.04	4.94	0.74	0.86	5.18	10.35
3	0.063	n=1	4.94	n=1	63.14		
4	0.031	7.37	4.94	4.26	19.47	21.82	43.64
5	0.031	0.00	4.94	0.00	20.33	20.92	41.85
6.1	0.033	6.06	4.94	2.29	15.19	17.24	34.48
6.2	0.036	8.33	4.94	3.40	7.48	12.70	25.41
7.1	0.044	n=1	4.94	n=1	13.08		
7.2	0.041	n=1	4.94	n=1	5.37		
8	0.028	10.10	4.94	7.14	28.04	31.04	62.09
9	0.044	9.67	4.94	6.84	13.74	18.80	37.59
10	0.046	4.75	4.94	3.36	19.31	20.77	41.53
11	0.040	0.88	4.94	0.62	3.51	6.15	12.31
12	0.039	1.26	4.94	0.89	0.00	5.18	10.35
13	0.035	n=1	4.94	n=1	9.42		
14	0.045	n=1	4.94	n=1	14.60		
15	0.039	4.23	4.94	2.99	0.51	7.18	14.35
D-Aile/L-Ile rpHPLC		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.026	5.19	5.71	1.64	33.84	34.75	69.49
2	0.039	1.04	5.71	0.74	0.00	5.85	11.69
3	0.063	n=1	5.71	n=1	61.74		
4							
5							
6.1							
6.2							
7.1							
7.2							
8	0.028	10.10	5.71	7.14	28.66	31.73	63.46
9	0.044	9.67	5.71	6.84	12.76	18.32	36.64
10	0.046	4.75	5.71	3.36	18.29	20.02	40.05
11	0.040	0.88	5.71	0.62	2.62	6.37	12.74
12	0.039	1.26	5.71	0.89	0.86	5.97	11.95
13	0.035	n=1	5.71	n=1	10.20		
14	0.045	n=1	5.71	n=1	13.62		
15	0.039	4.23	5.71	2.99	1.37	7.83	15.65

[4] = σ is the standard deviation of submitted results, expressed as a relative % i.e.; $CV\% = (\sigma/\bar{x}) \times 100$ (see Section 4).
[5] =$u(\hat{X})$ is the uncertainty of the assigned value $(\hat{X})$ expressed as a relative % i.e.; $RSU_{\hat{X}}\% = (u(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = $u(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\hat{x}}\% = (u(\bar{x})/\bar{x}) \times 100$ (see Section 4).
[7] = Relative bias expressed as a % i.e.; $Bias\% = (\bar{x} - \hat{X}/\hat{X}) \times 100$

Table 6.2: Estimation of Relative Standard Uncertainty, Combined and Expanded Uncertainty Estimations for Individual Laboratories (continued).

laboratory number	mean result	Std uncertainty contributions Precision[4]	Bias components[5,6,7]			Combined & Expanded uncertainties	
Leu D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.064	5.96	5.22	1.89	9.94	12.85	25.70
2							
3							
4							
5							
6.1	0.040	2.50	5.22	0.94	31.26	31.80	63.61
6.2	0.047	8.51	5.22	3.47	19.23	21.94	43.88
7.1	0.044	n=1	5.22	n=1	24.38		
7.2	0.043	n=1	5.22	n=1	26.10		
8	0.053	0.00	5.22	0.00	8.92	10.33	20.66
9	0.063	2.47	5.22	1.74	7.99	10.01	20.01
10	0.066	0.42	5.22	0.30	12.69	13.73	27.45
11	0.061	2.42	5.22	1.71	4.63	7.58	15.16
12	0.062	0.29	5.22	0.20	6.76	8.54	17.08
13	0.035	n=1	5.22	n=1	39.17		
14	0.100	n=1	5.22	n=1	72.14		
15	0.058	0.43	5.22	0.31	0.00	5.24	10.49
Leu D/L rpHPLC		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1	0.064	5.96	2.75	1.89	2.98	7.45	14.90
2							
3							
4							
5							
6.1							
6.2							
7.1							
7.2							
8	0.053	0.00	2.75	0.00	14.68	14.94	29.87
9	0.063	2.47	2.75	1.74	1.15	4.24	8.48
10	0.066	0.42	2.75	0.30	5.55	6.22	12.43
11	0.061	2.42	2.75	1.71	1.99	4.50	9.01
12	0.062	0.29	2.75	0.20	0.00	2.77	5.54
13	0.035	n=1	2.75	n=1	43.02		
14	0.100	n=1	2.75	n=1	61.25		
15	0.058	0.43	2.75	0.31	6.33	6.92	13.84

[4] = σ is the standard deviation of submitted results, expressed as a relative % i.e.; $CV\% = (\sigma/\bar{x}) \times 100$ (see Section 4).
[5] =$u(\hat{X})$ is the uncertainty of the assigned value $(\hat{X})$ expressed as a relative % i.e.; $RSU_{\hat{X}}\% = (u(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = $u(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\hat{x}}\% = (u(\bar{x})/\bar{x}) \times 100$ (see Section 4).
[7] = Relative bias expressed as a % i.e.; $Bias\% = (\bar{x} - \hat{X}/\hat{X}) \times 100$

Table 6.2: Estimation of Relative Standard Uncertainty, Combined and Expanded Uncertainty Estimations for Individual Laboratories (continued).

laboratory number	mean result	Std uncertainty contributions: Precision[4]	Bias components[5,6,7]			Combined & Expanded uncertainties	
Try D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
1							
2							
3							
4							
5							
6.1							
6.2							
7.1							
7.2							
8							
9	0.085	0.13	2.61	0.09	11.60	11.89	23.77
10	0.084	1.85	2.61	1.31	11.05	11.58	23.15
11	0.076	n=1	2.61	n=1	0.00		
12	0.078	0.59	2.61	0.42	2.22	3.50	7.00
13	0.071	n=1	2.61	n=1	5.70		
14	0.074	n=1	2.61	n=1	1.82		
15	0.072	0.49	2.61	0.35	4.65	5.36	10.73

[4] = σ is the standard deviation of submitted results, expressed as a relative % i.e.; $CV\% = (\sigma/\bar{x}) \times 100$ (see Section 4).
[5] =$\mathrm{u}(\hat{\mathrm{X}})$ is the uncertainty of the assigned value $(\hat{\mathrm{X}})$ expressed as a relative % i.e.; $RSU_{\hat{\mathrm{X}}}\% = (\mathrm{u}(\hat{\mathrm{X}})/\hat{\mathrm{X}}) \times 100$ (see Section 5)
[6] = $\mathrm{u}(\bar{\mathrm{x}})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\hat{x}}\% = (\mathrm{u}(\bar{x})/\bar{x}) \times 100$ (see Section 4).
[7] = Relative bias expressed as a % i.e.; $Bias\% = (\bar{x} - \hat{\mathrm{X}}/\hat{\mathrm{X}}) \times 100$

Figure 6.2: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Aspartic acid / Asparagine** D/L Values in Ostrich Egg Shell (A) Test Material

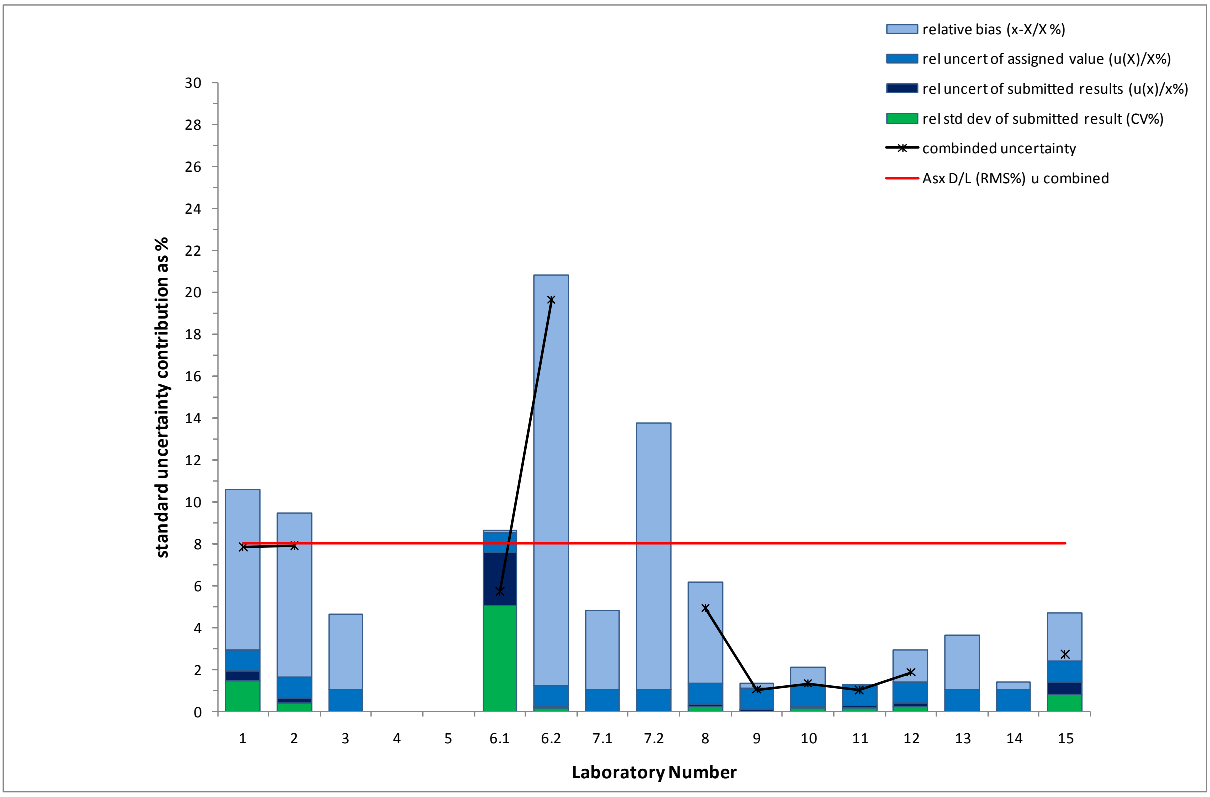


Figure 6.3: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Aspartic acid / Asparagine** D/L Values in Ostrich Egg Shell (A) Test Material

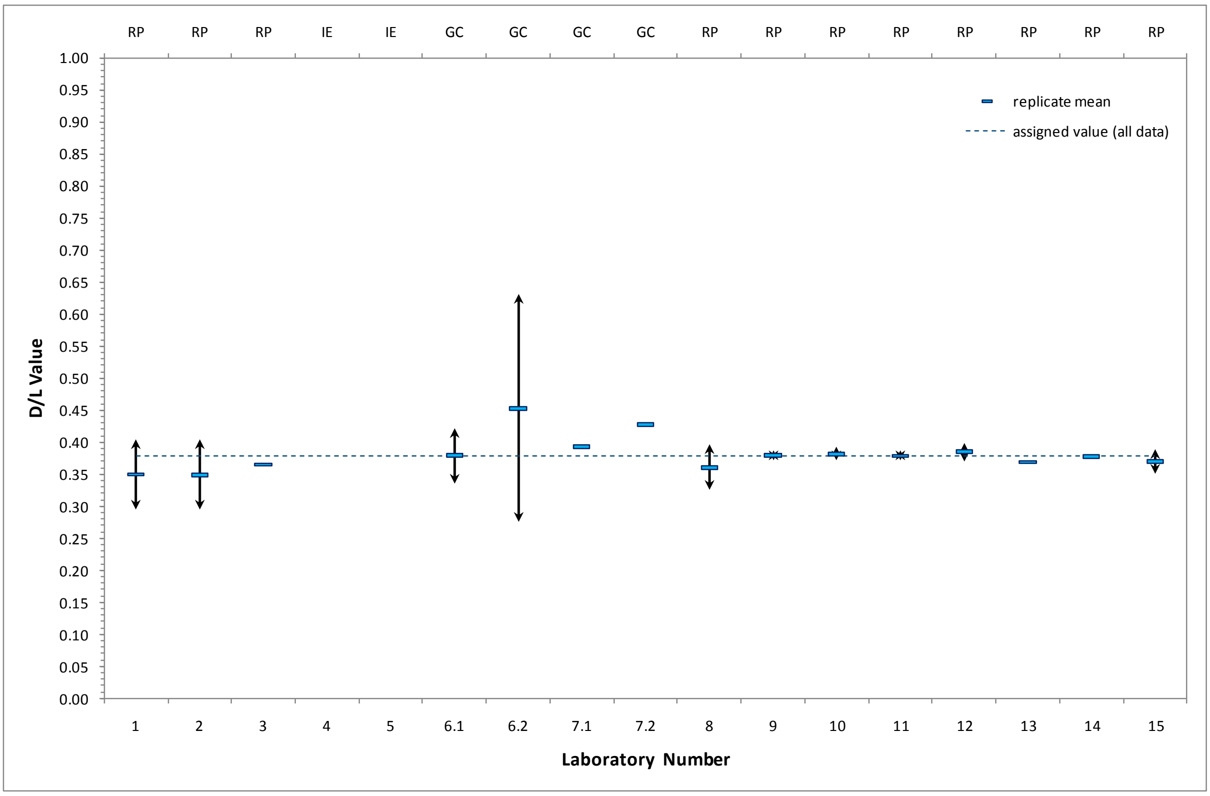


Figure 6.4: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Aspartic acid / Asparagine rpHPLC** D/L Values in Ostrich Egg Shell (A) Test Material

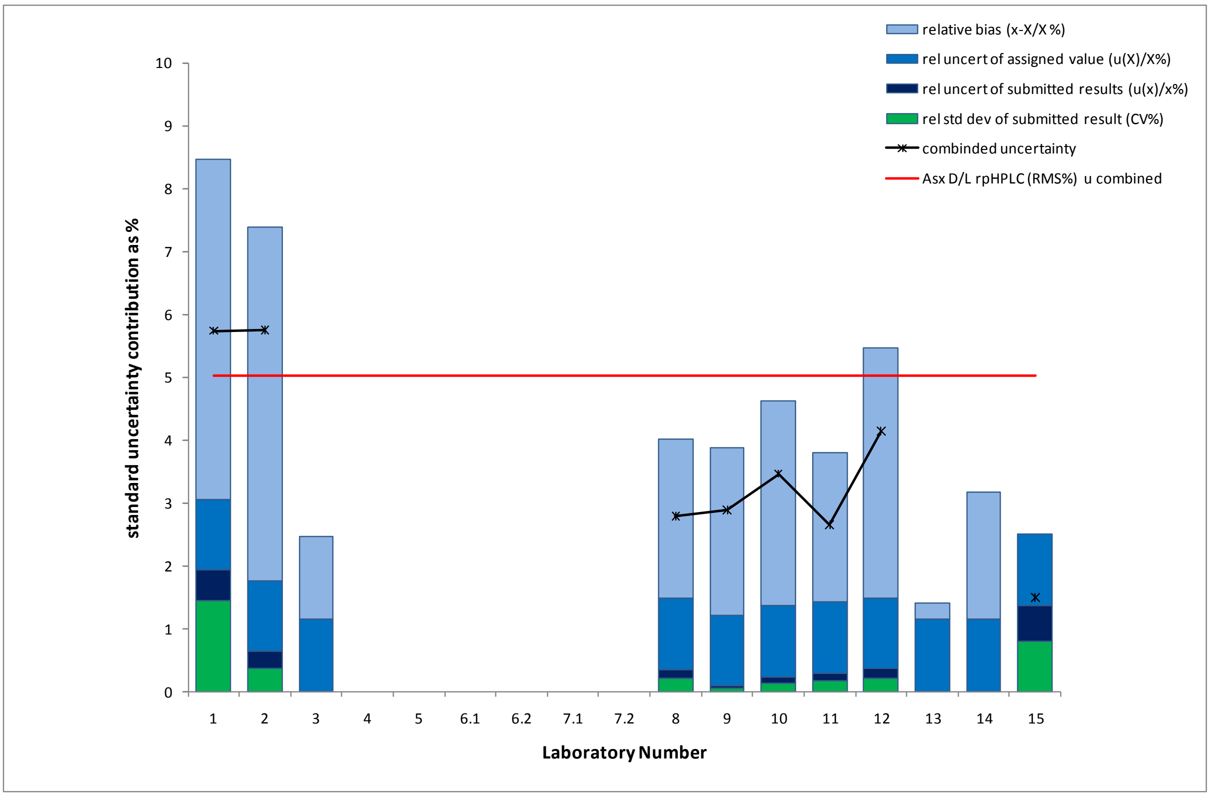


Figure 6.5: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Aspartic acid / Asparagine rpHPLC** D/L Values in Ostrich Egg Shell (A) Test Material

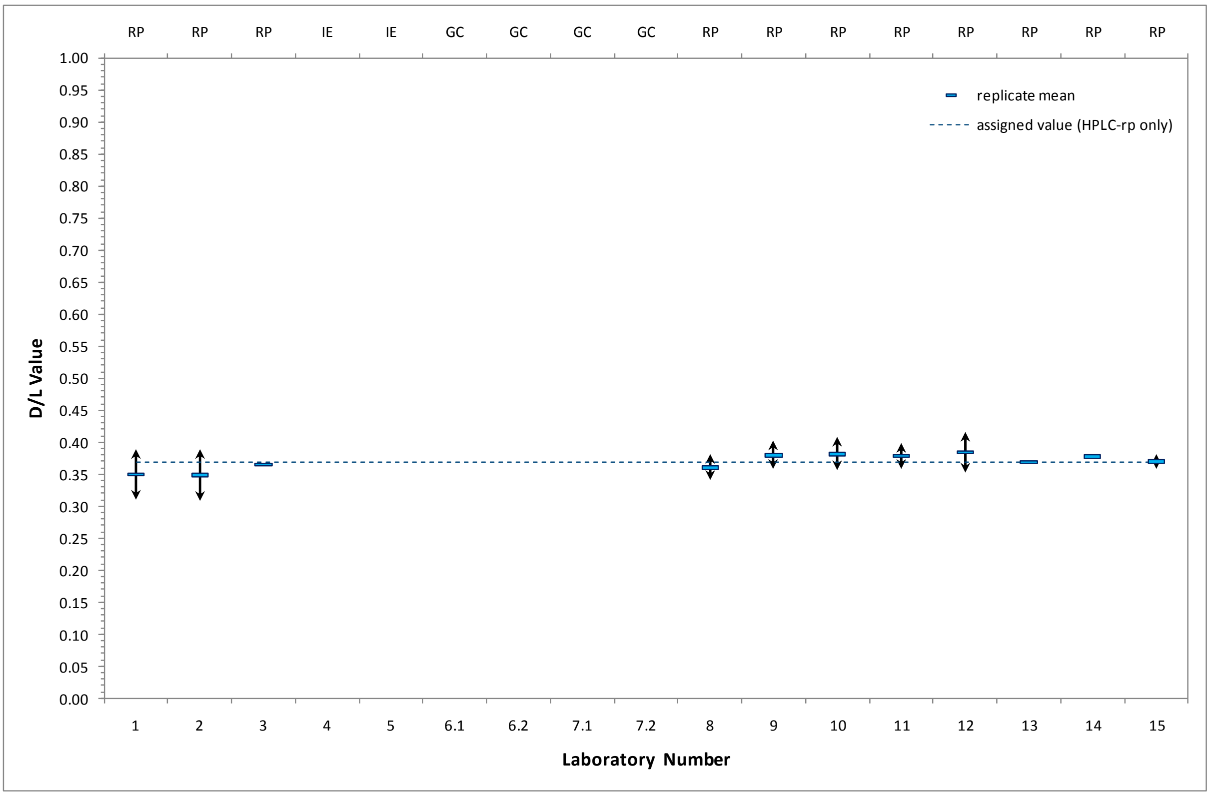


Figure 6.6: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Glutamic acid / Glutamine** D/L Values in Ostrich Egg Shell (A) Test Material

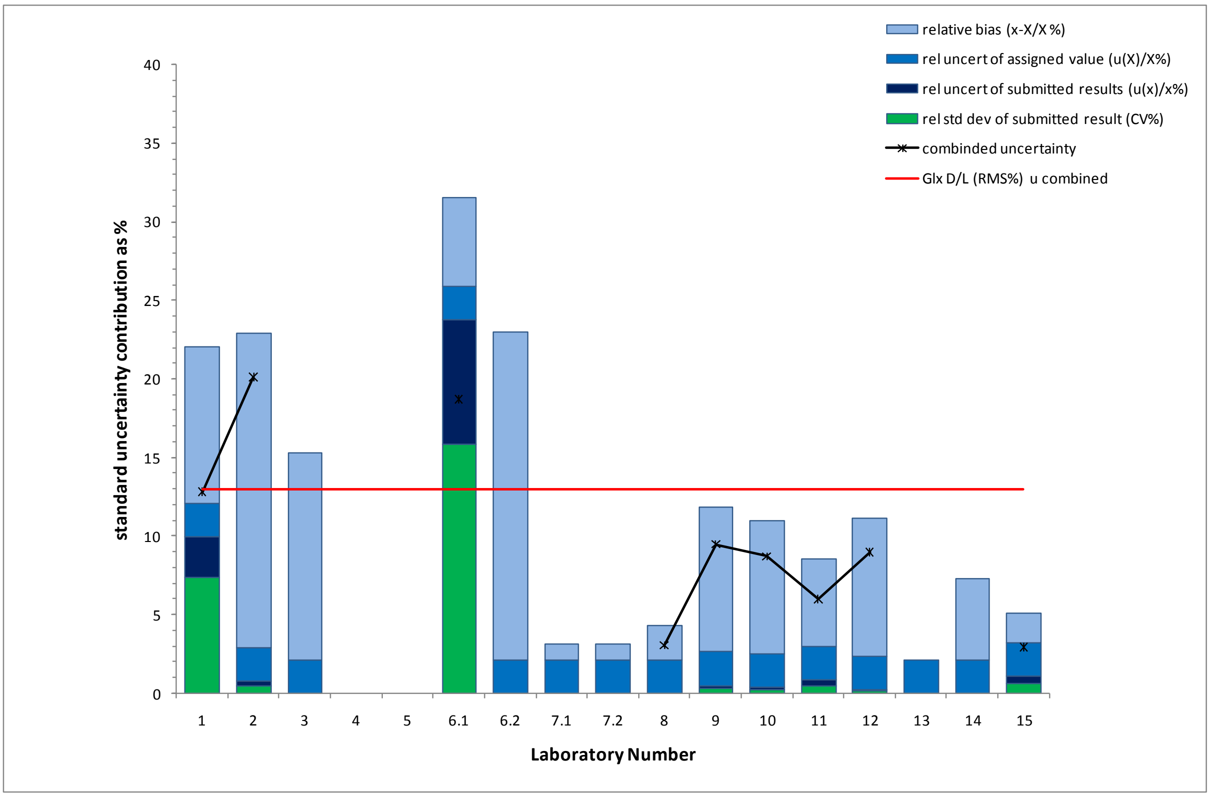


Figure 6.7: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Glutamic acid / Glutamine** D/L Values in Ostrich Egg Shell (A) Test Material

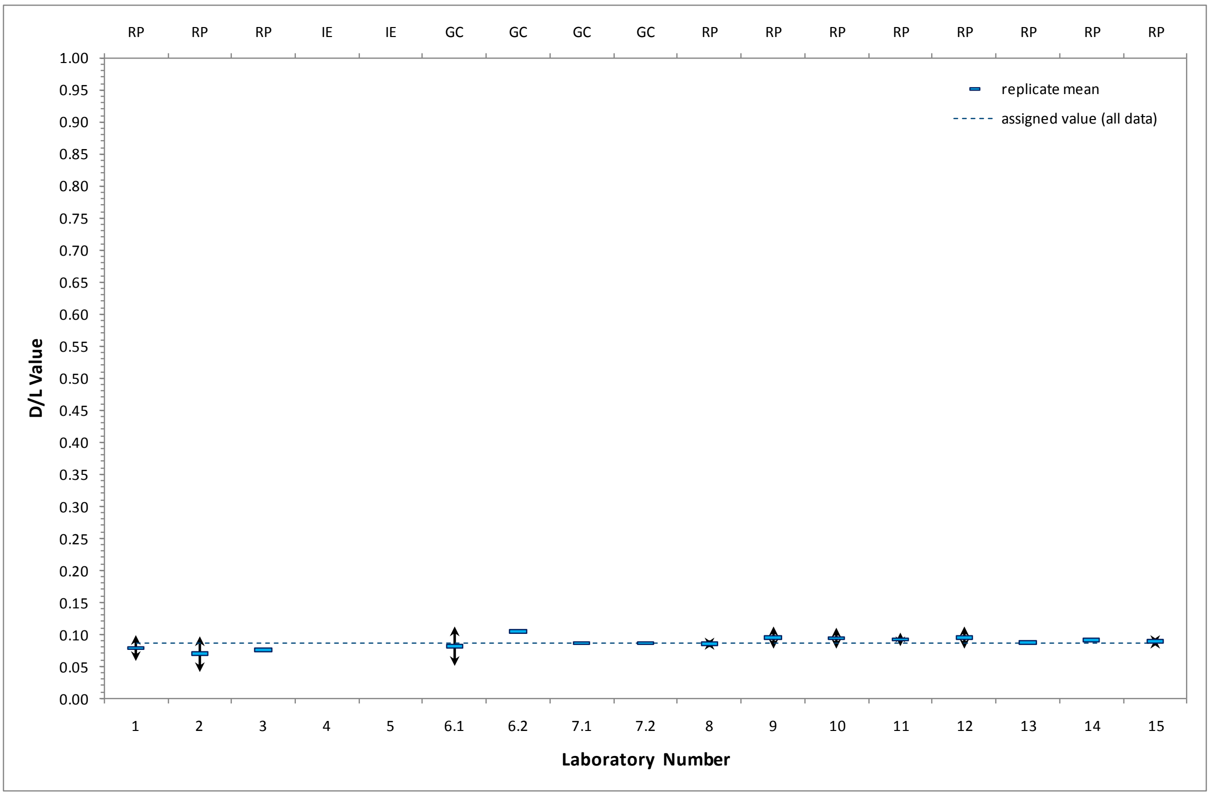


Figure 6.8: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Glutamic acid /Glutamine rpHPLC** D/L Values in Ostrich Egg Shell (A) Test Material

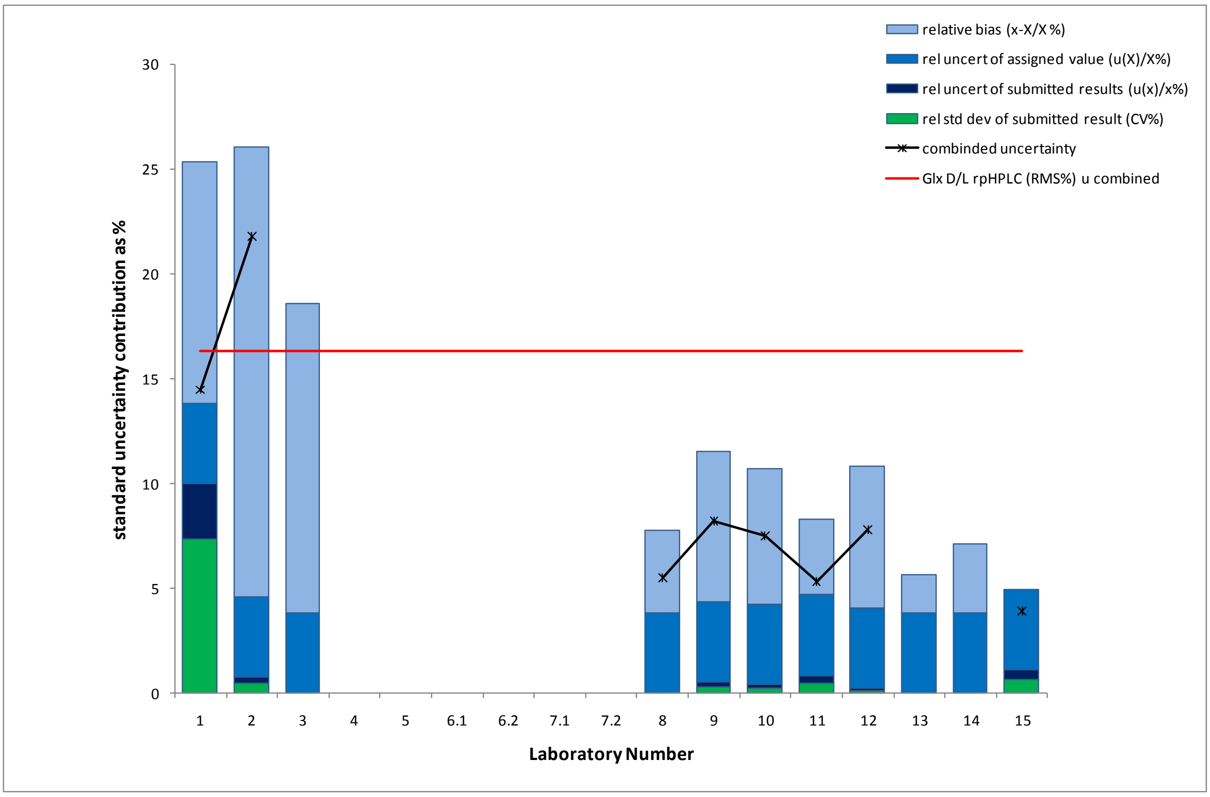


Figure 6.9: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Glutamic acid / Glutamine rpHPLC** D/L Values in Ostrich Egg Shell (A) Test Material

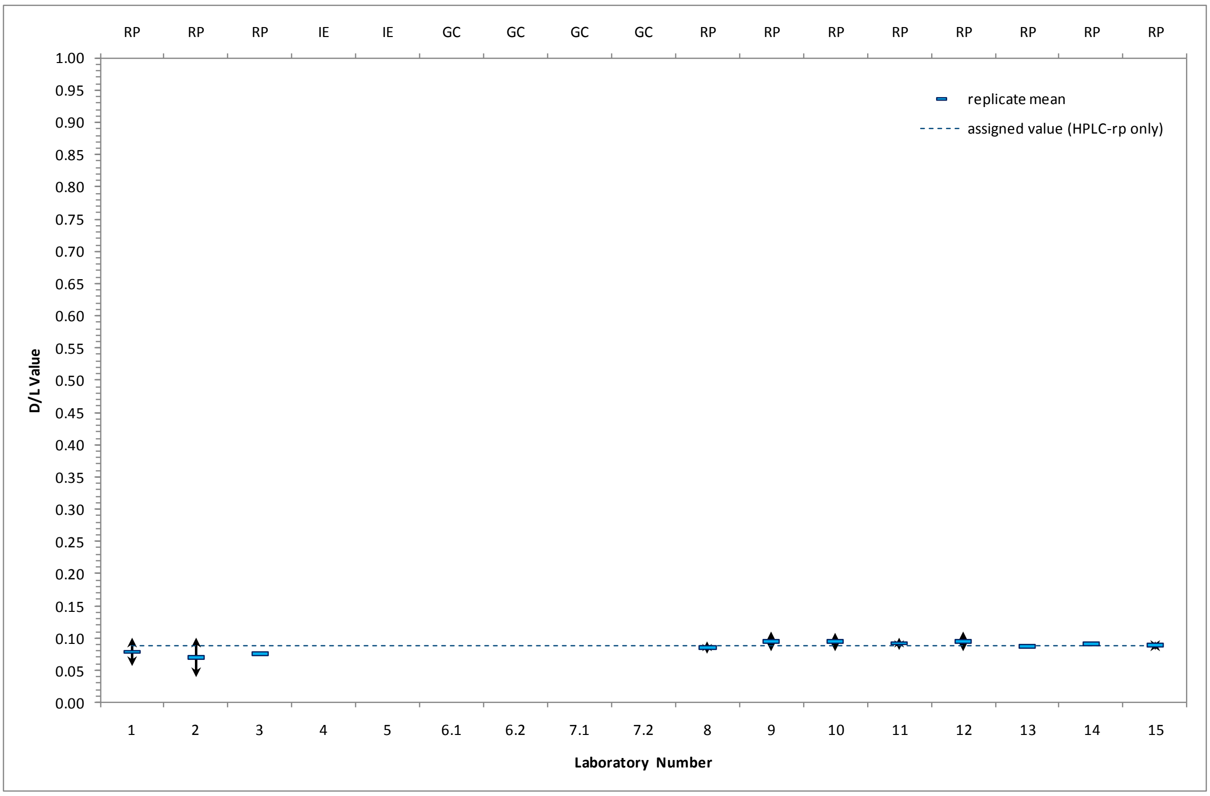


Figure 6.10: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Serine** D/L Values in Ostrich Egg Shell (A) Test Material

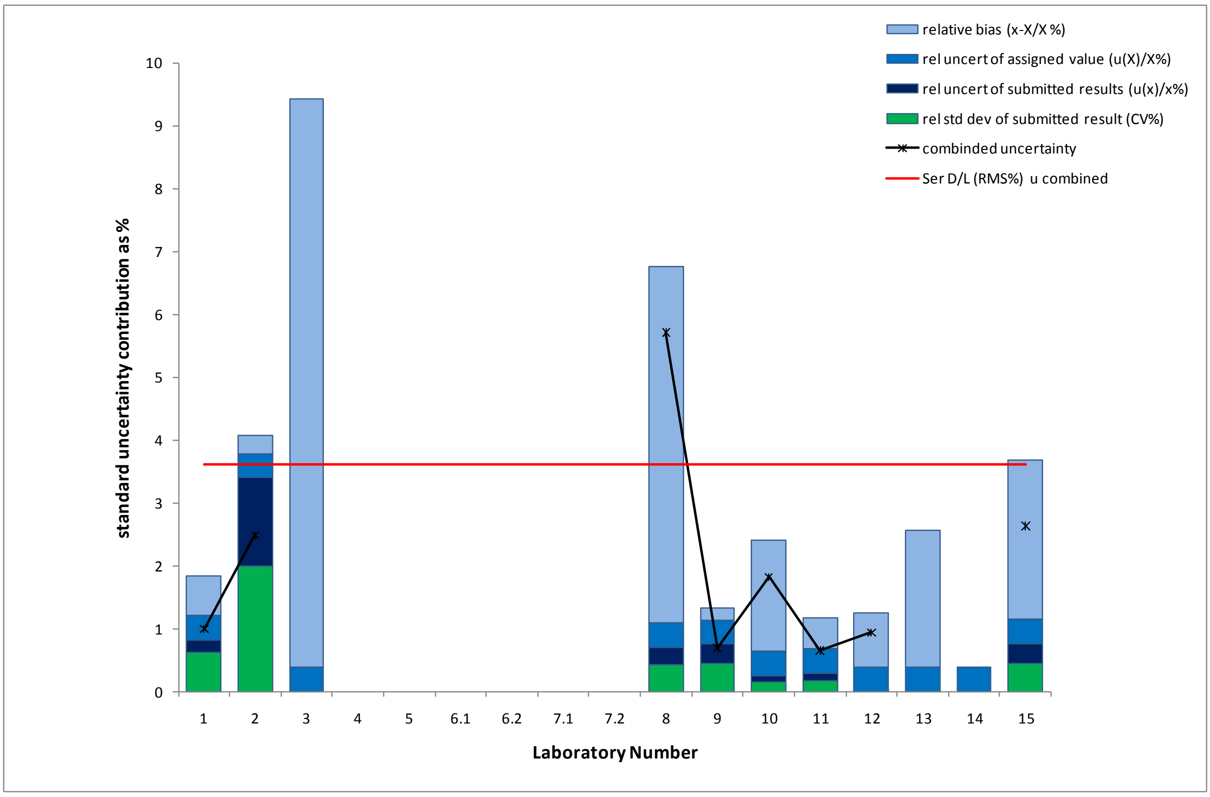


Figure 6.11: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Serine** D/L Values in Ostrich Egg Shell (A) Test Material

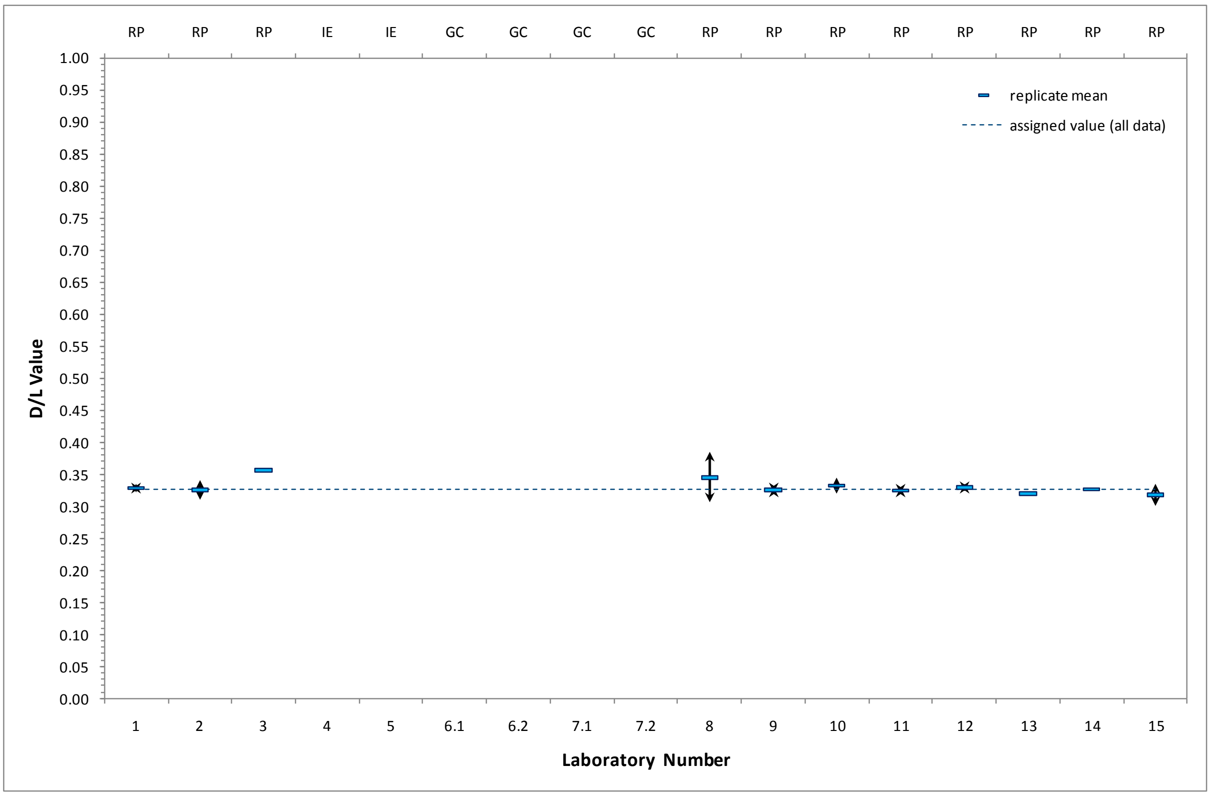


Figure 6.12: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Arginine** D/L Values in Ostrich Egg Shell (A) Test Material

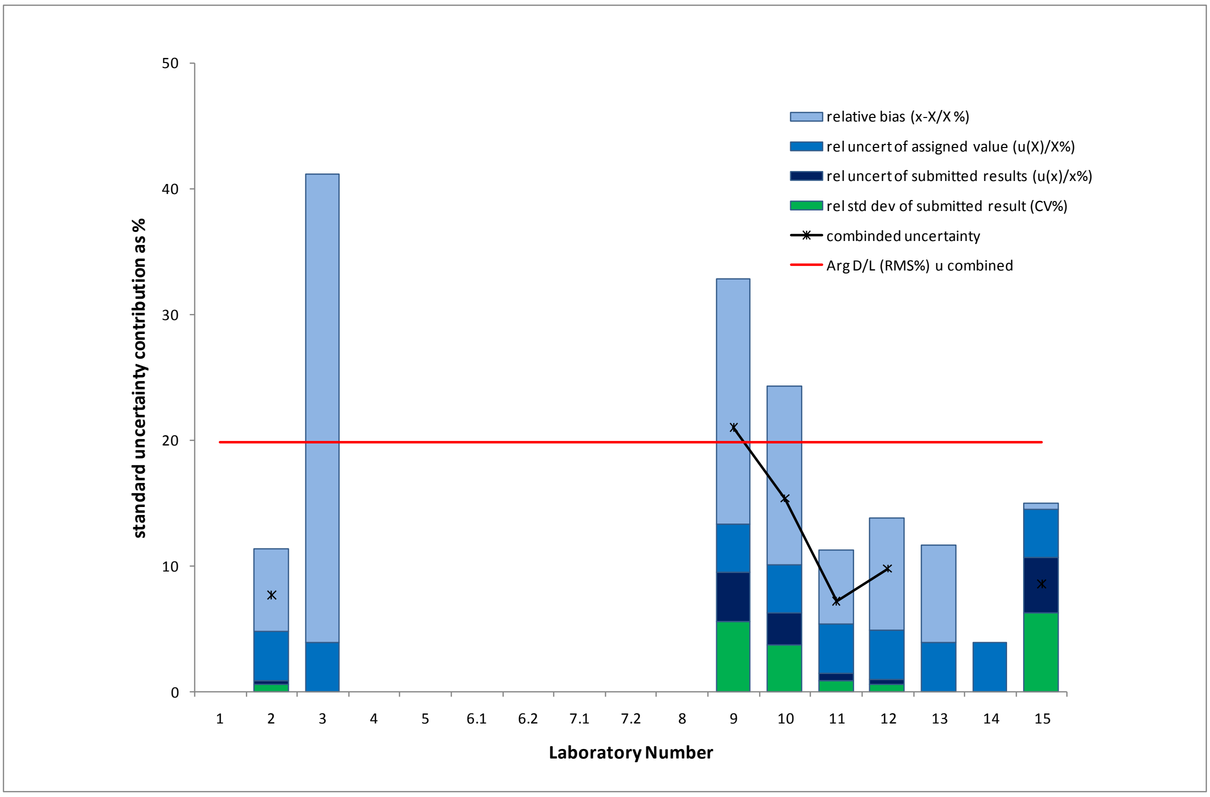


Figure 6.13: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Arginine** D/L Values in Ostrich Egg Shell (A) Test Material

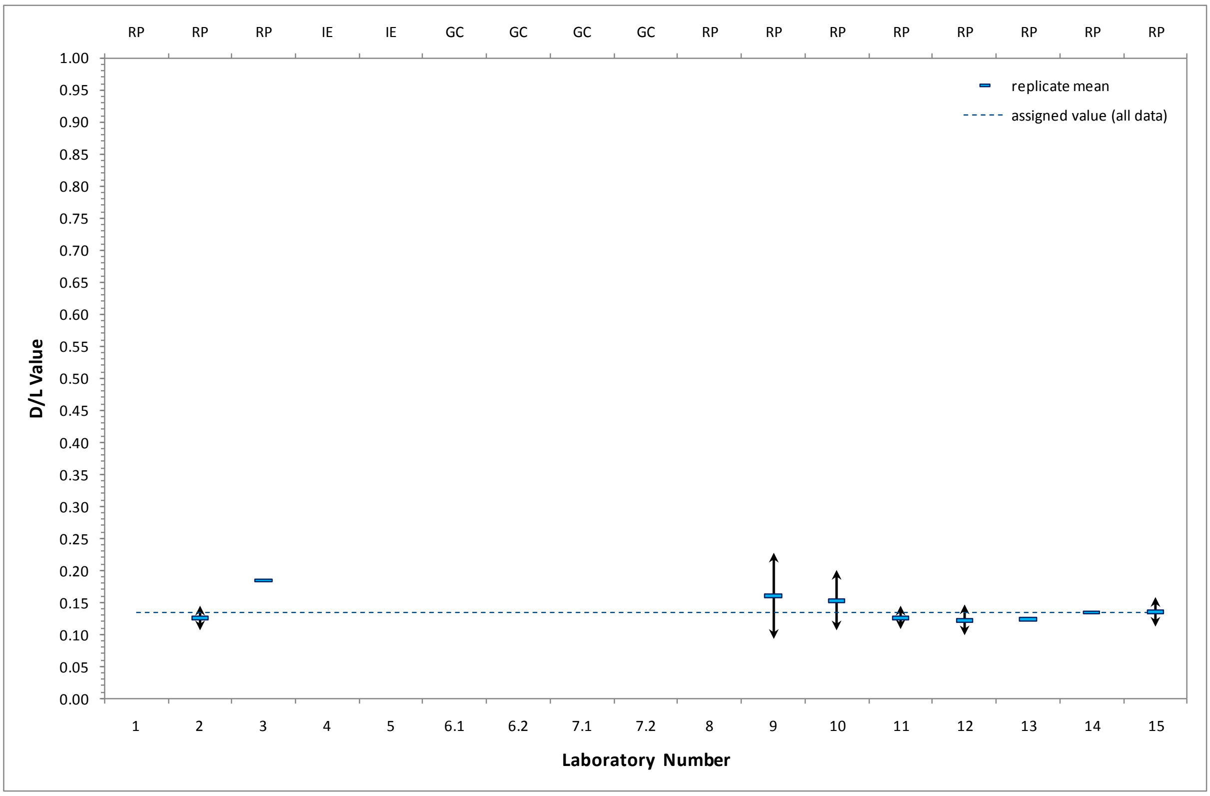


Figure 6.14: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Alanine** D/L Values in Ostrich Egg Shell (A) Test Material

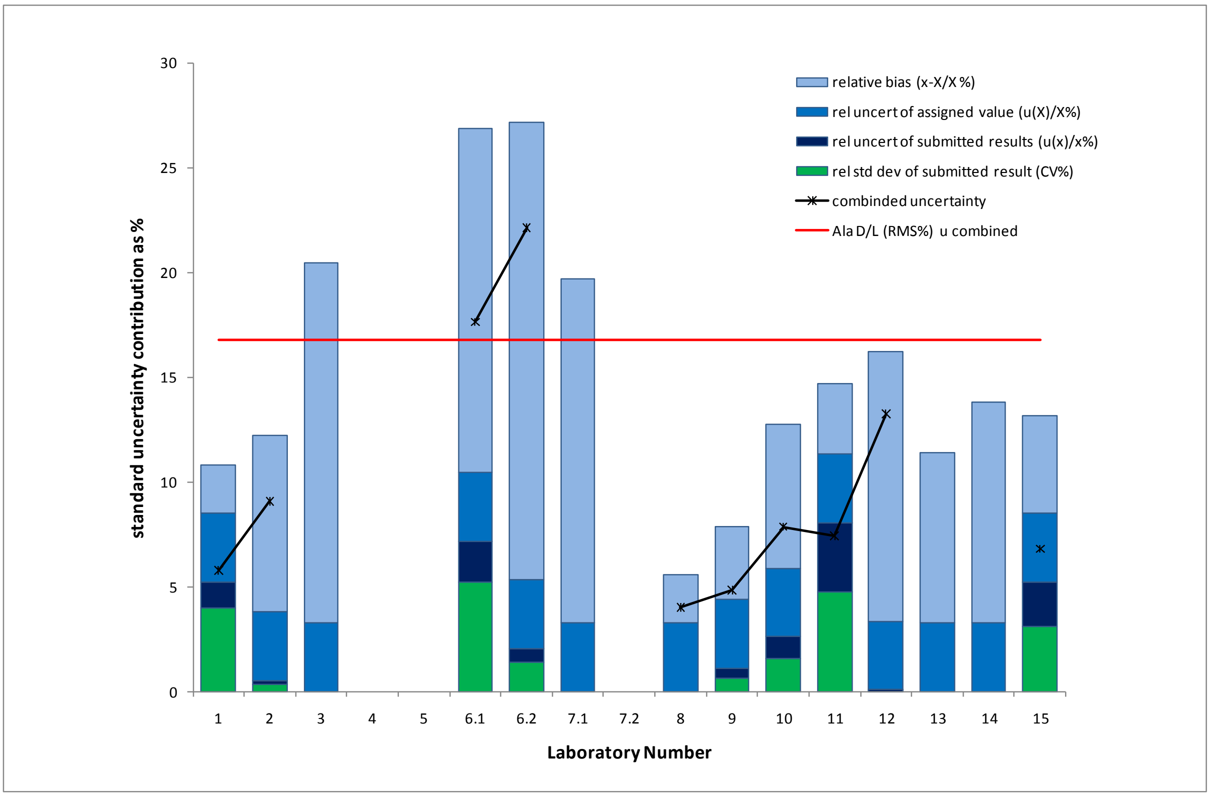


Figure 6.15: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Alanine** D/L Values in Ostrich Egg Shell (A) Test Material

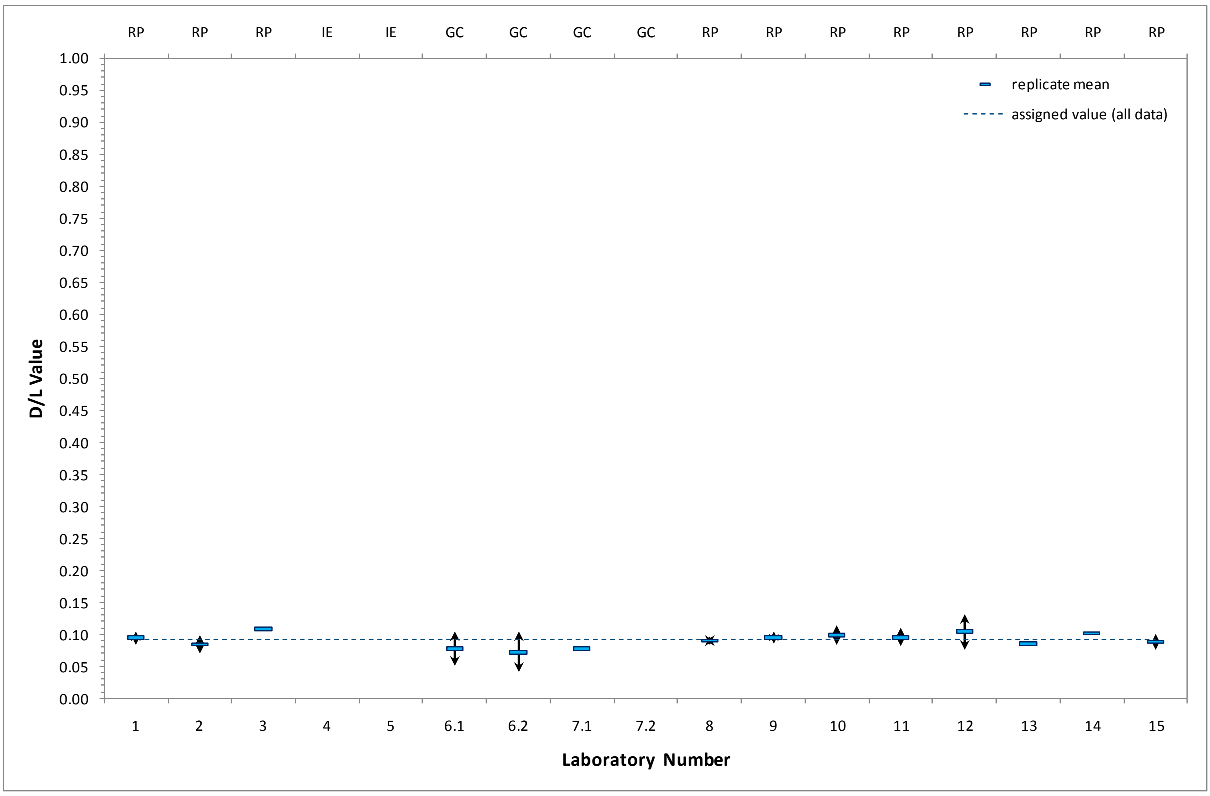


Figure 6.16: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Alanine (rpHPLC)** D/L Values in Ostrich Egg Shell (A) Test Material

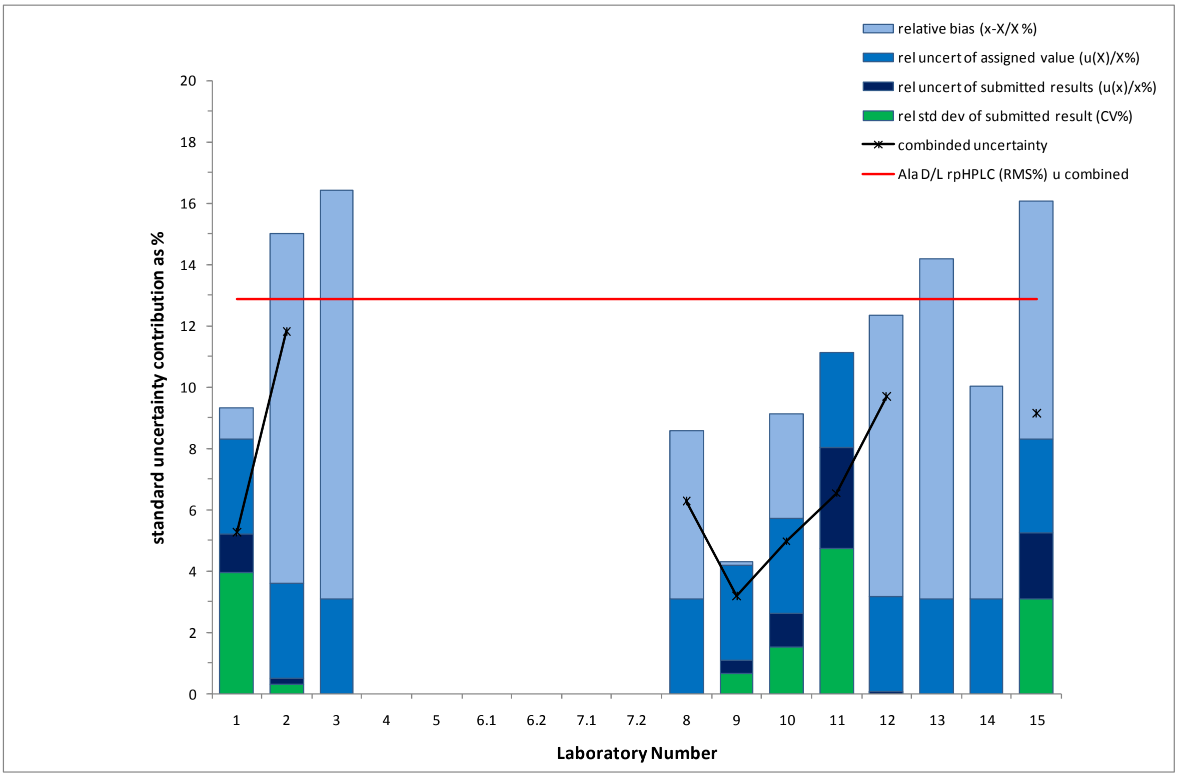


Figure 6.17: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Alanine (rpHPLC)** D/L Values in Ostrich Egg Shell (A) Test Material

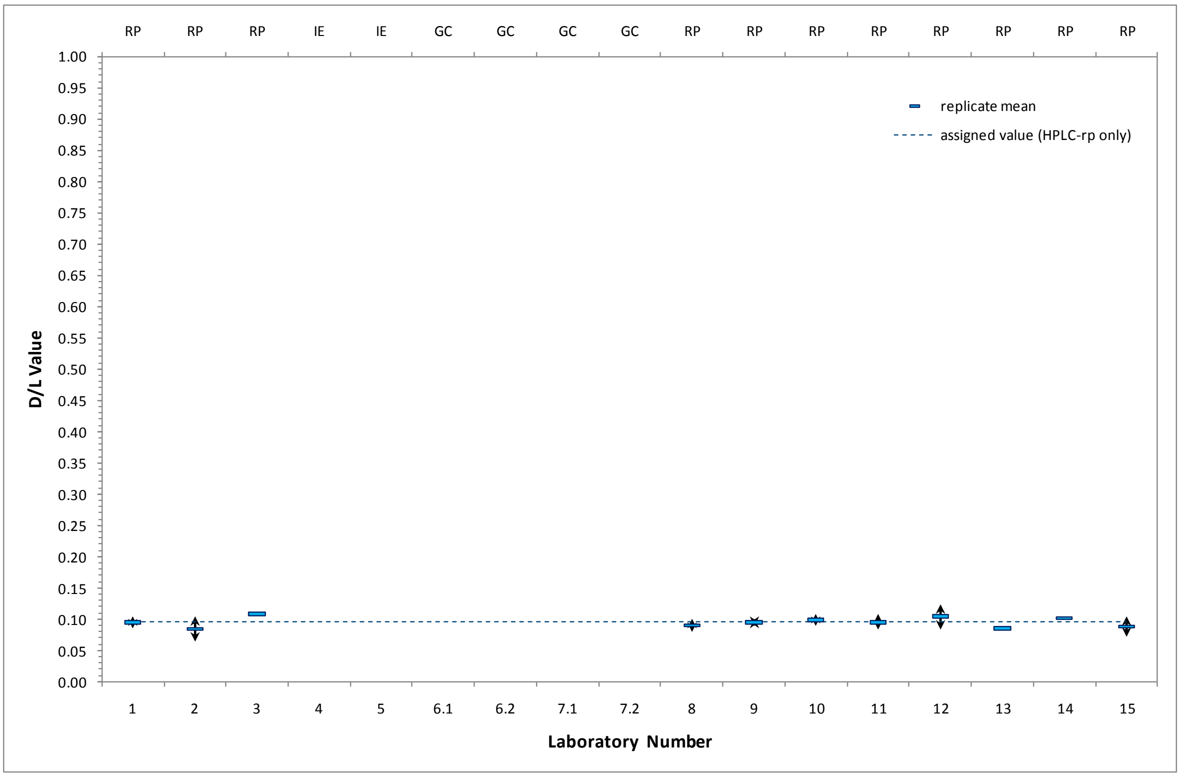


Figure 6.18: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Valine** D/L Values in Ostrich Egg Shell (A) Test Material

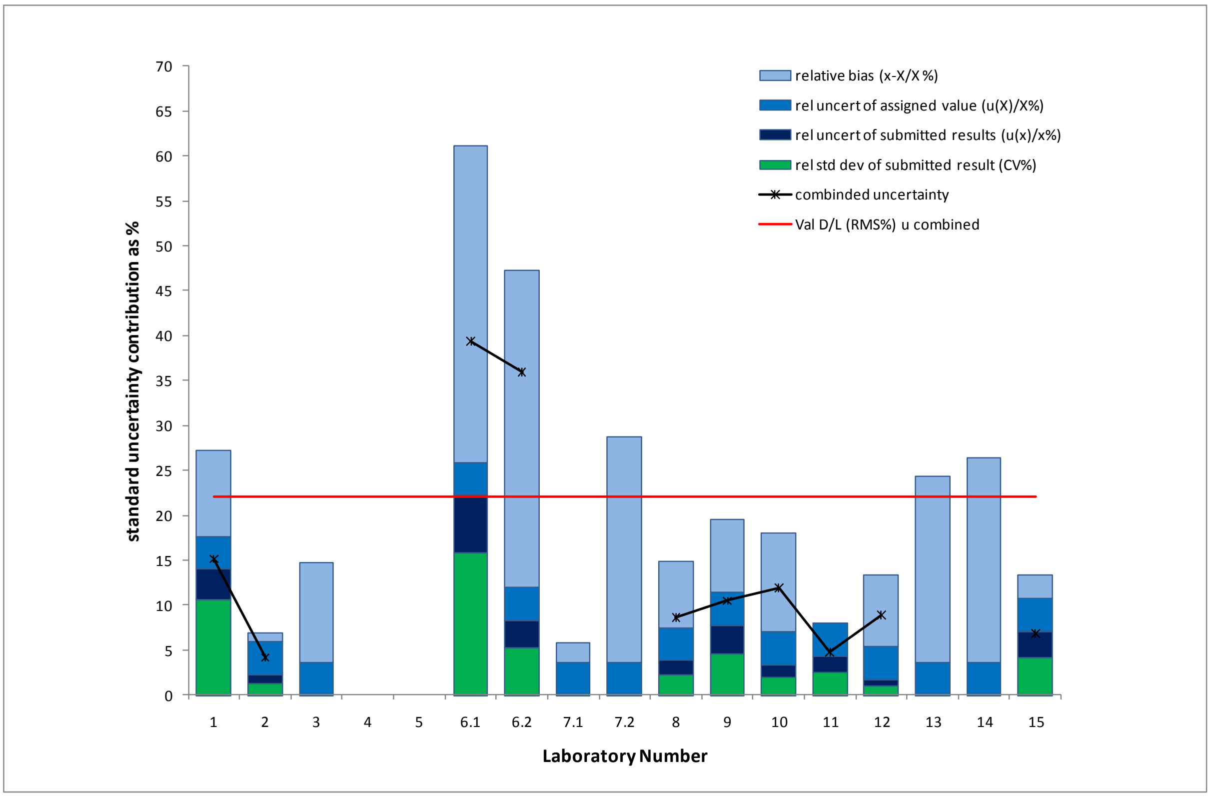


Figure 6.19: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Valine** D/L Values in Ostrich Egg Shell (A) Test Material

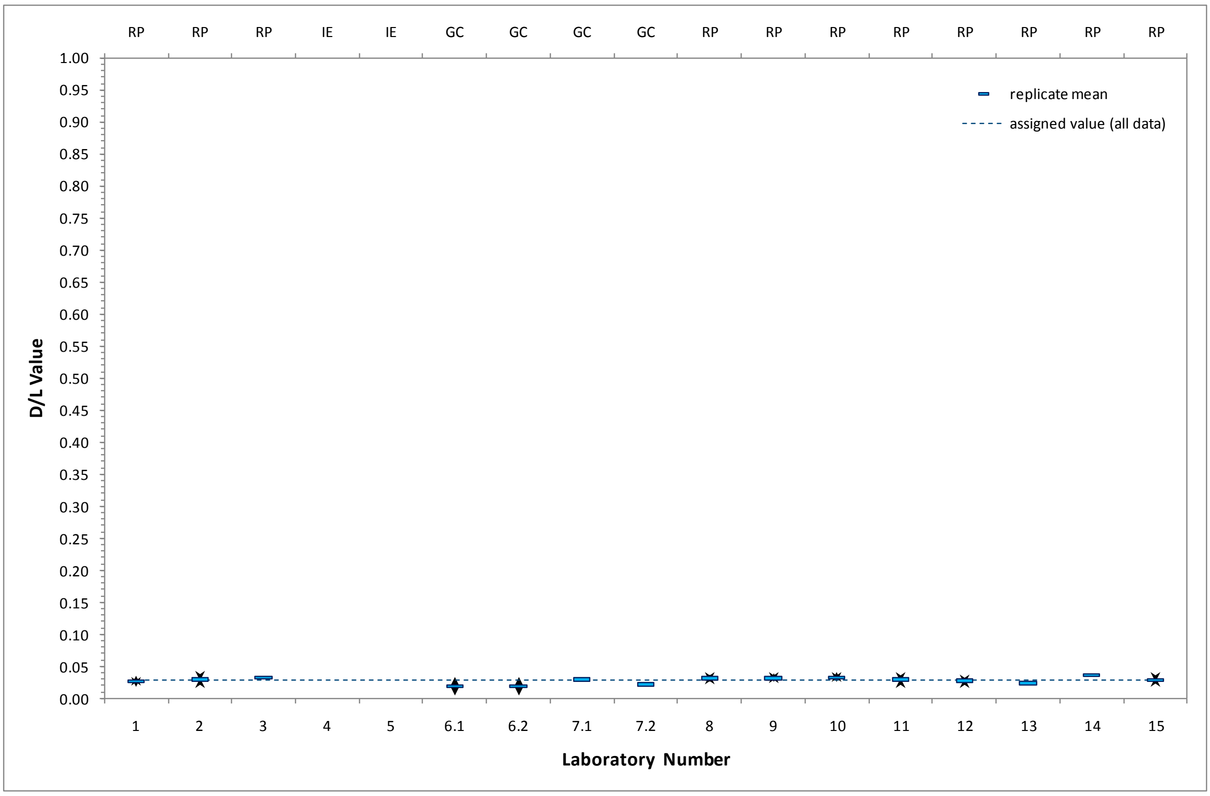


Figure 6.20: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Valine (rpHPLC)** D/L Values in Ostrich Egg Shell (A) Test Material

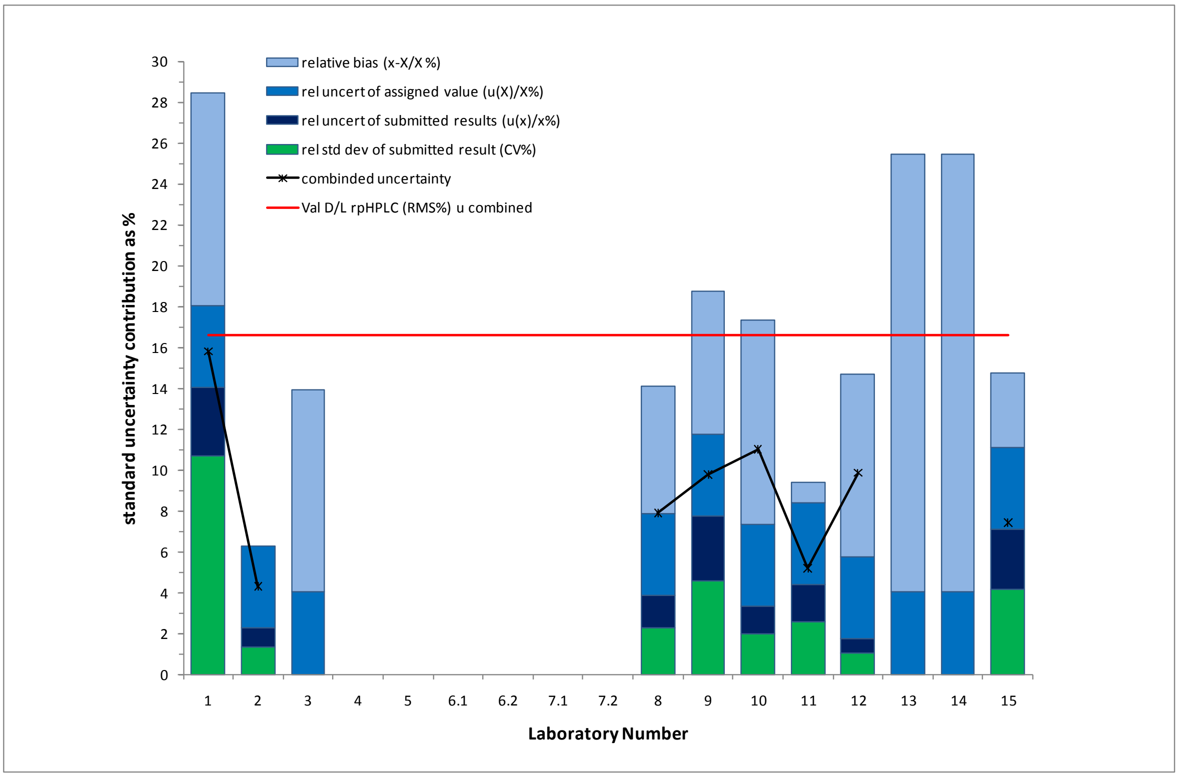


Figure 6.21: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Valine (rpHPLC)** D/L Values in Ostrich Egg Shell (A) Test Material

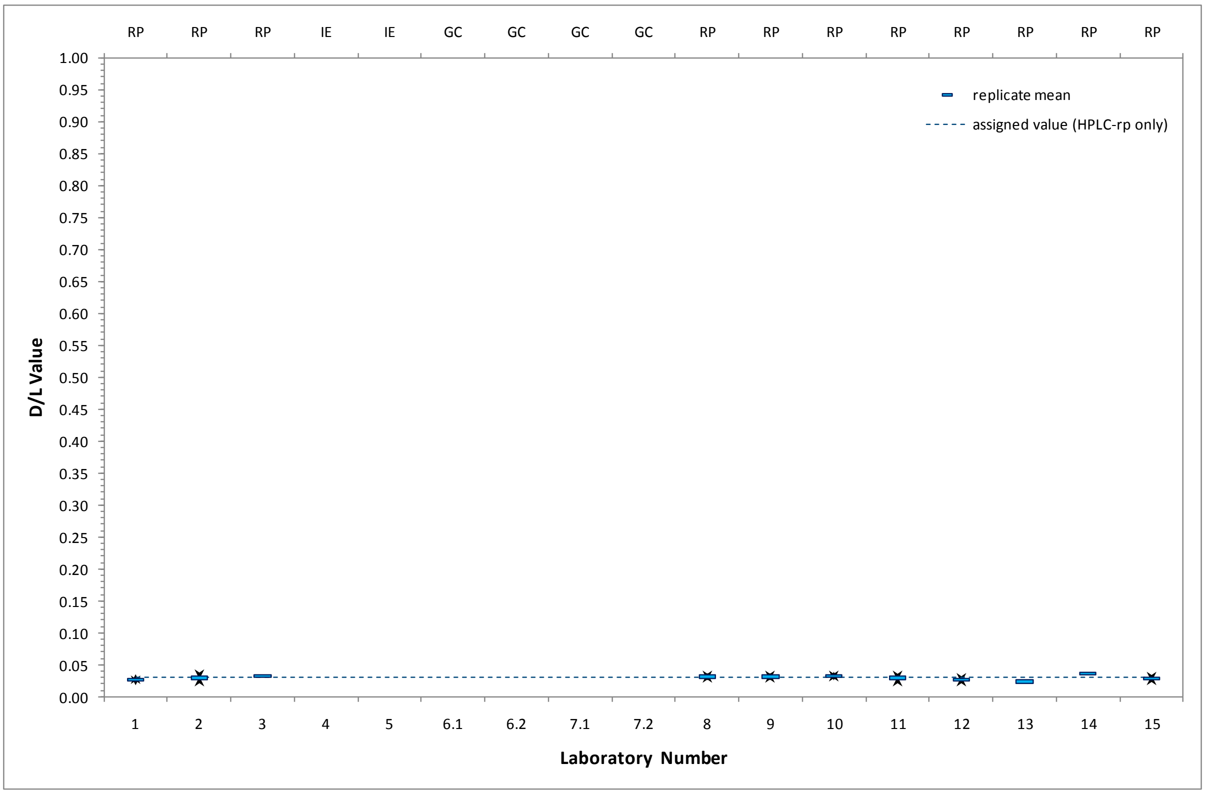


Figure 6.22: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Phenylalanine** D/L Values in Ostrich Egg Shell (A) Test Material

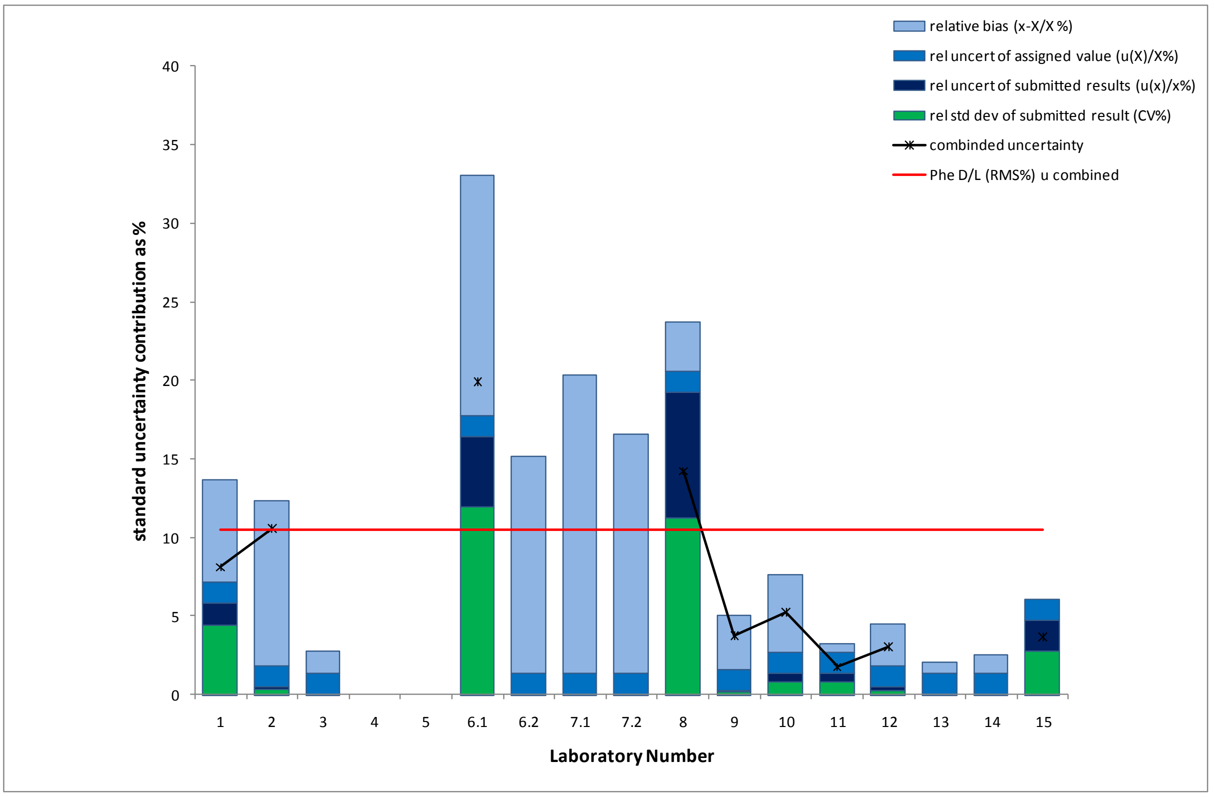


Figure 6.23: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Phenylalanine** D/L Values in Ostrich Egg Shell (A) Test Material

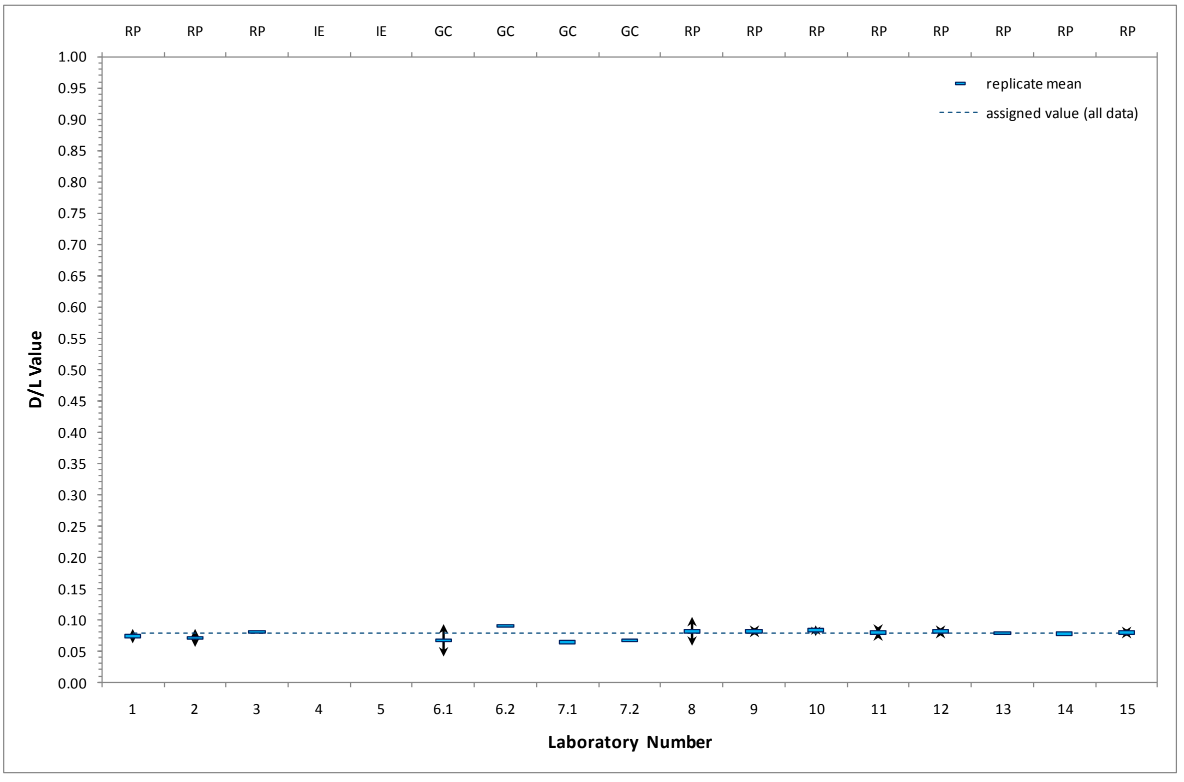


Figure 6.24: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Phenylalanine (rpHPLC)** D/L Values in Ostrich Egg Shell (A) Test Material

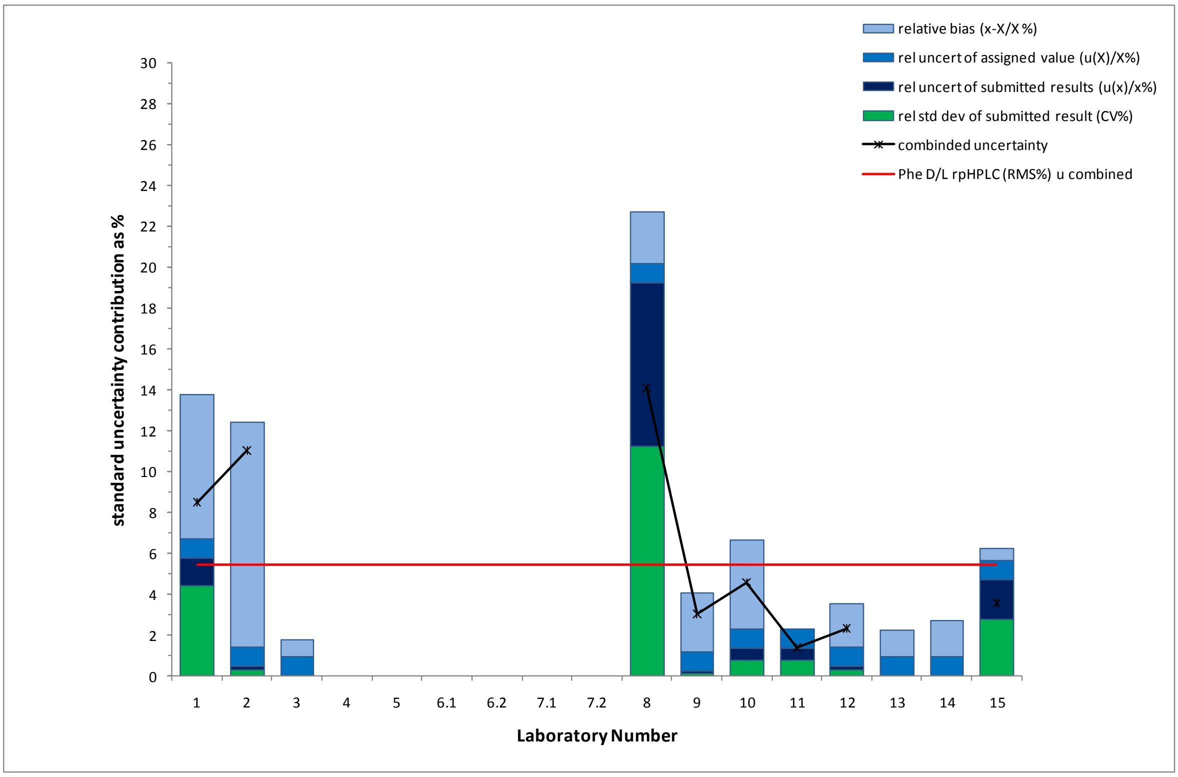


Figure 6.25: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Phenylalanine (rpHPLC)** D/L Values in Ostrich Egg Shell (A) Test Material

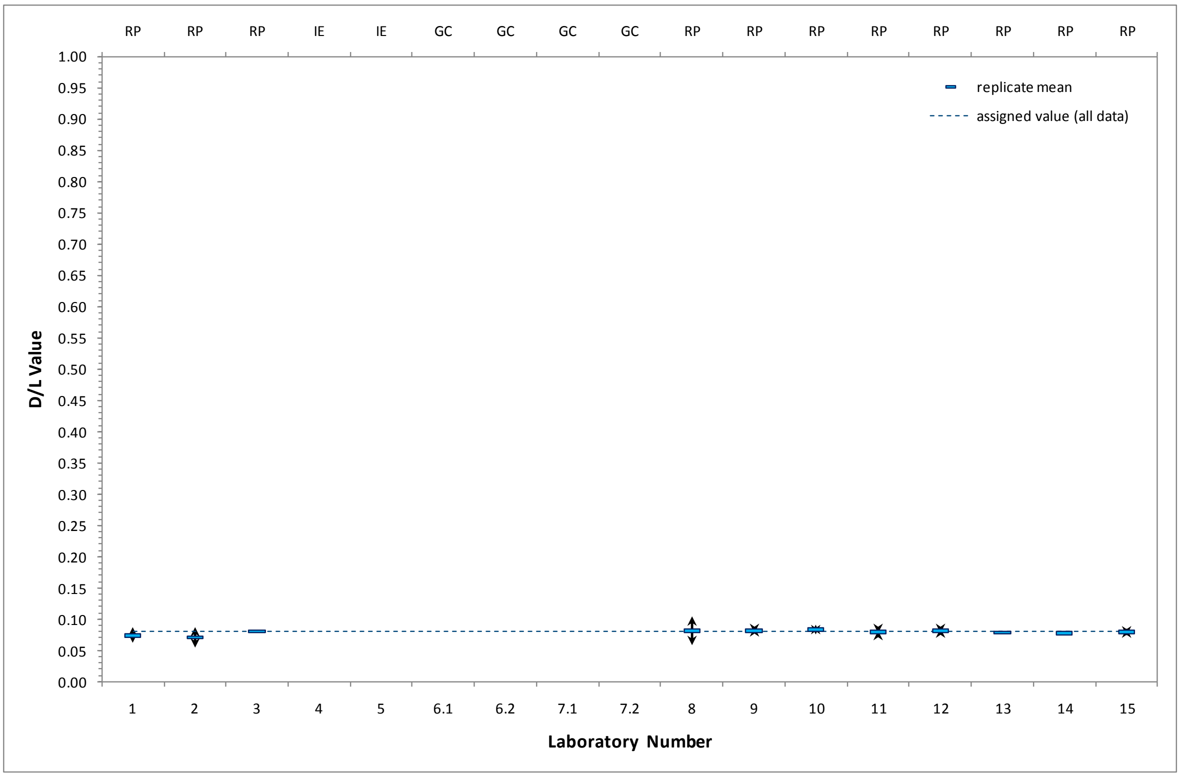


Figure 6.26: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **D-Alloisoleucine/L-Isoleucine** Values in Ostrich Egg Shell (A) Test Material

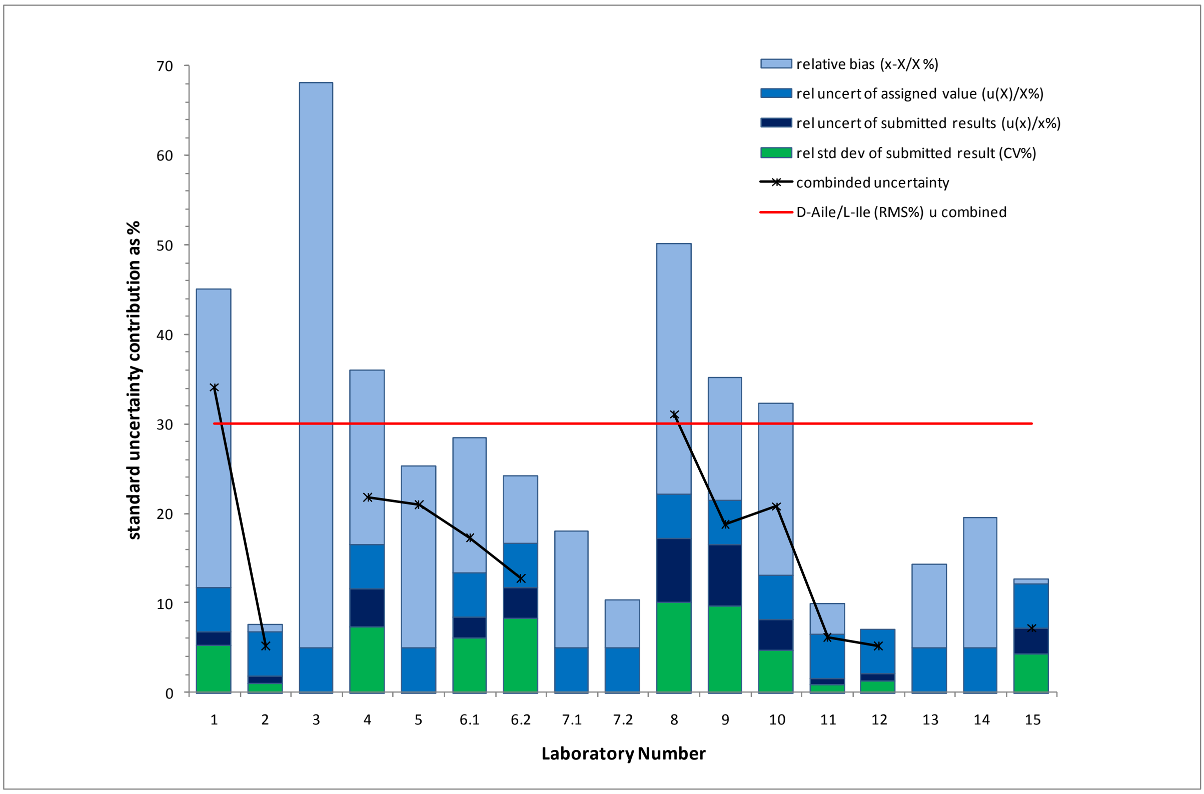


Figure 6.27: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **D-Alloisoleucine/L-Isoleucine** Values in Ostrich Egg Shell (A) Test Material

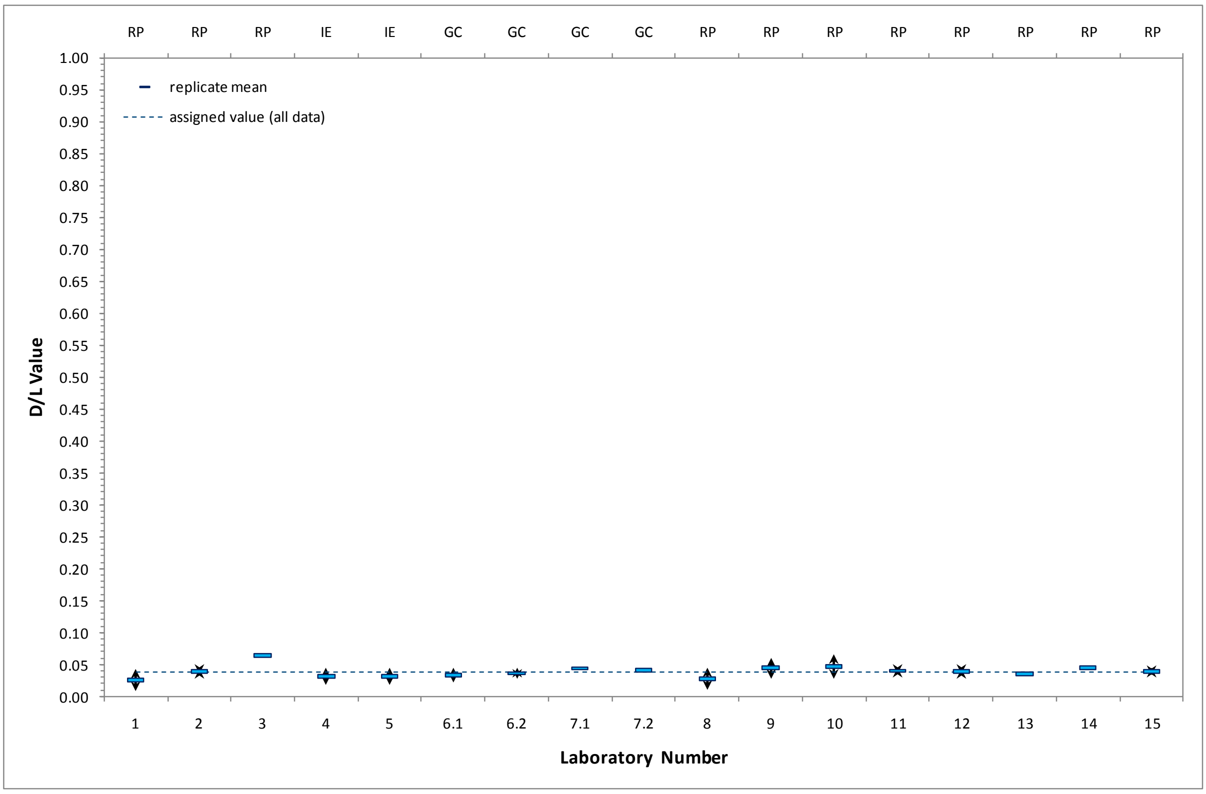


Figure 6.28: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **D-Alloisoleucine/L-Isoleucine rpHPLC** Values in Ostrich Egg Shell (A) Test Material

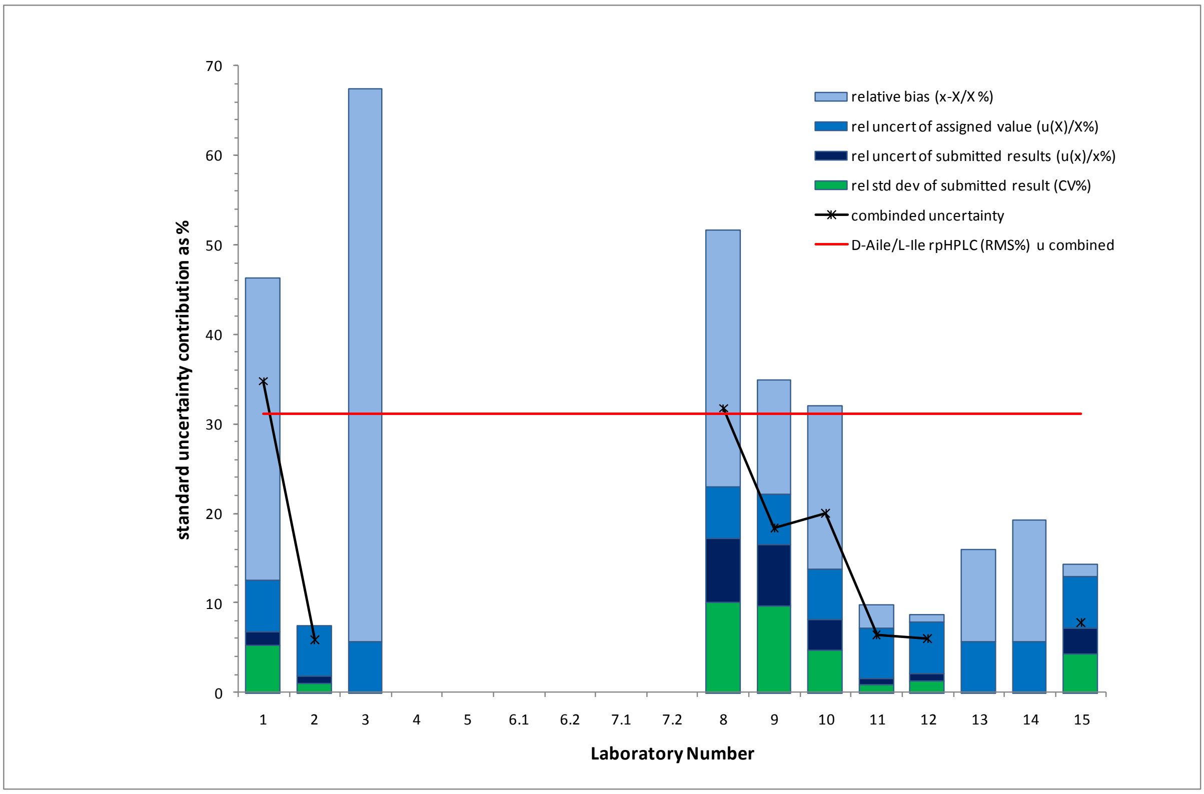


Figure 6.29: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **D-Alloisoleucine/L-Isoleucine rpHPLC** Values in Ostrich Egg Shell (A) Test Material

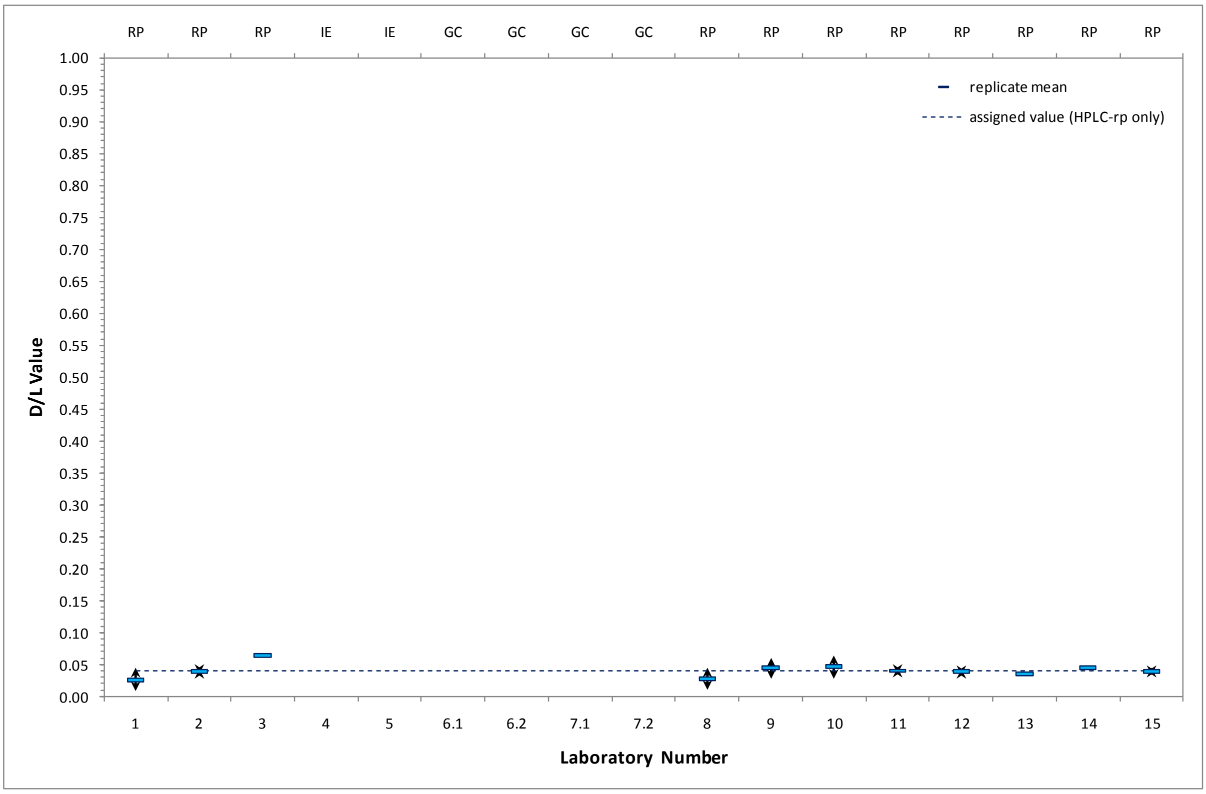


Figure 6.30: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Leucine** D/L Values in Ostrich Egg Shell (A) Test Material

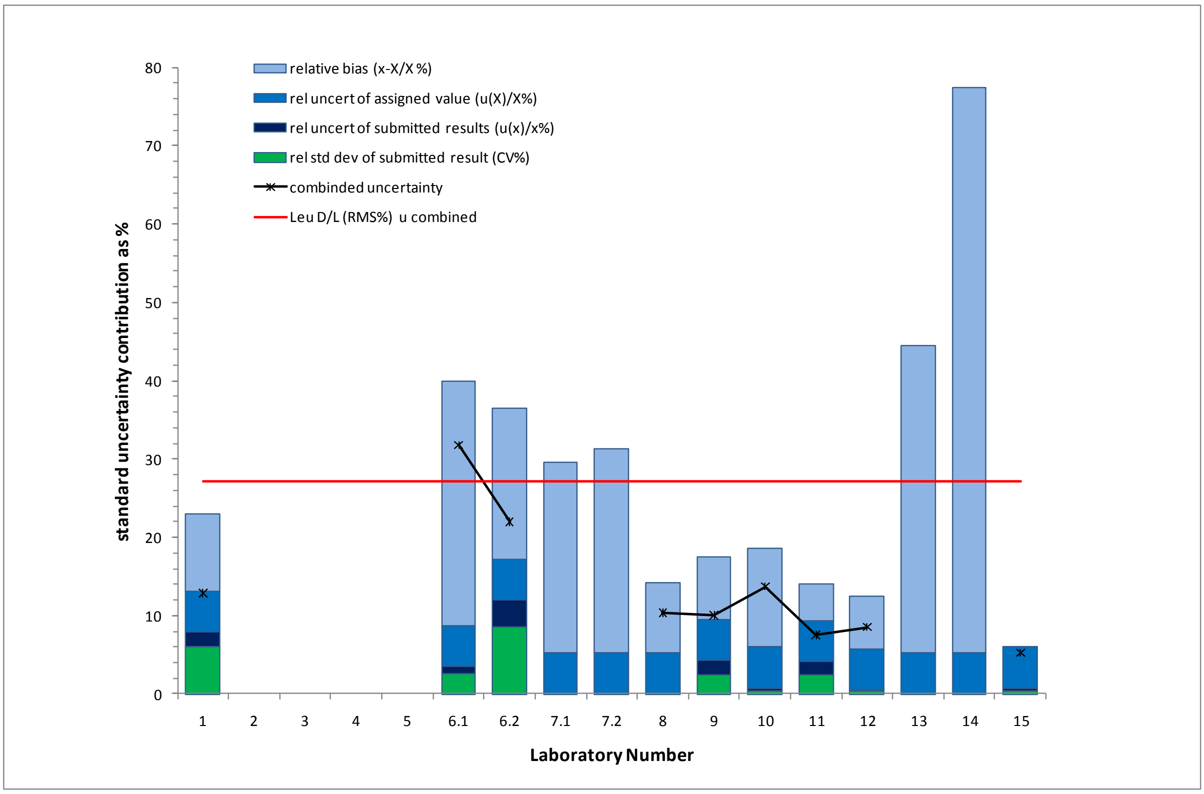


Figure 6.31: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Leucine** D/L Values in Ostrich Egg Shell (A) Test Material

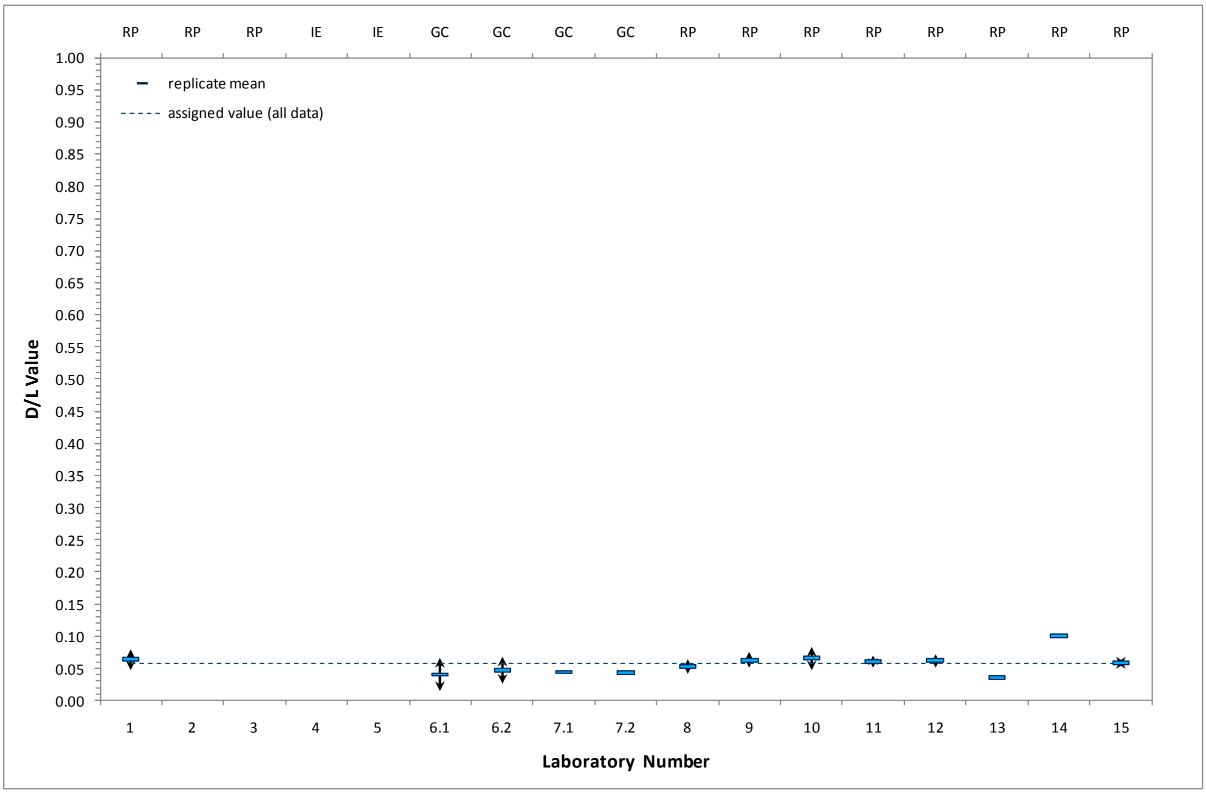


Figure 6.32: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Leucine rpHPLC** D/L Values in Ostrich Egg Shell (A) Test Material

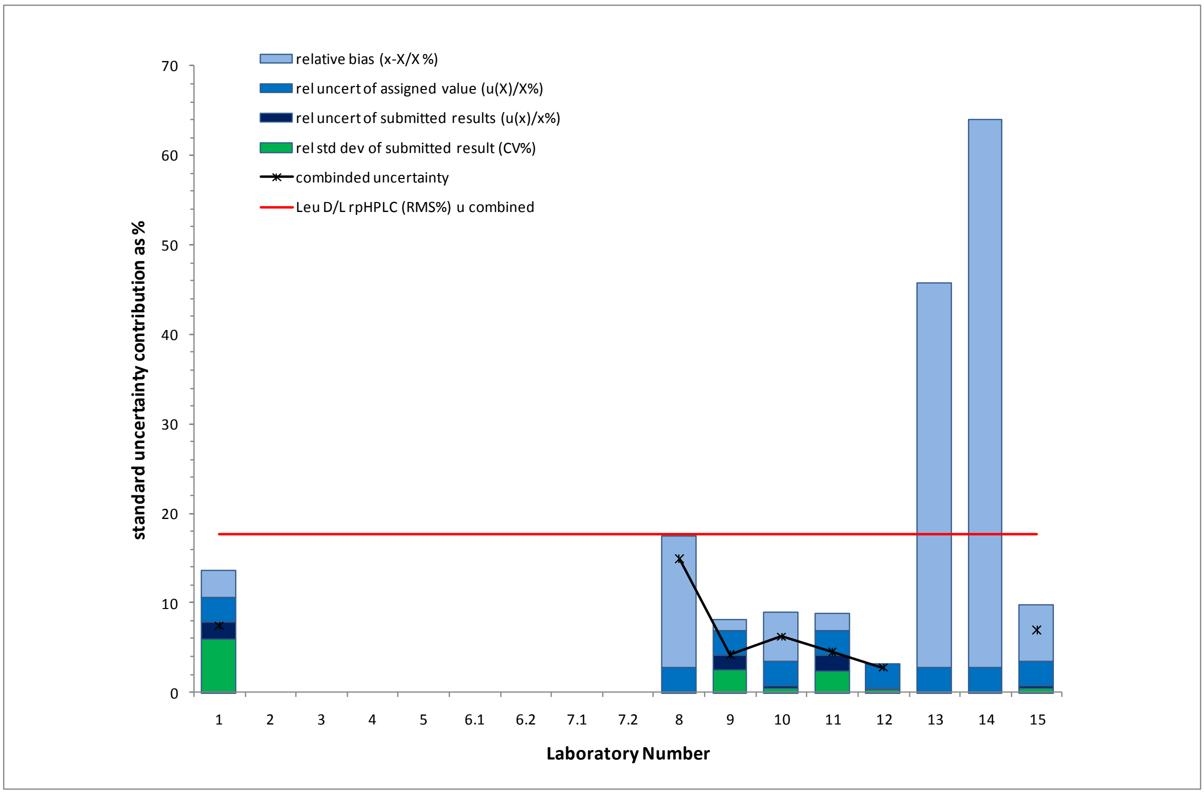


Figure 6.33: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Leucine rpHPLC** D/L Values in Ostrich Egg Shell (A) Test Material

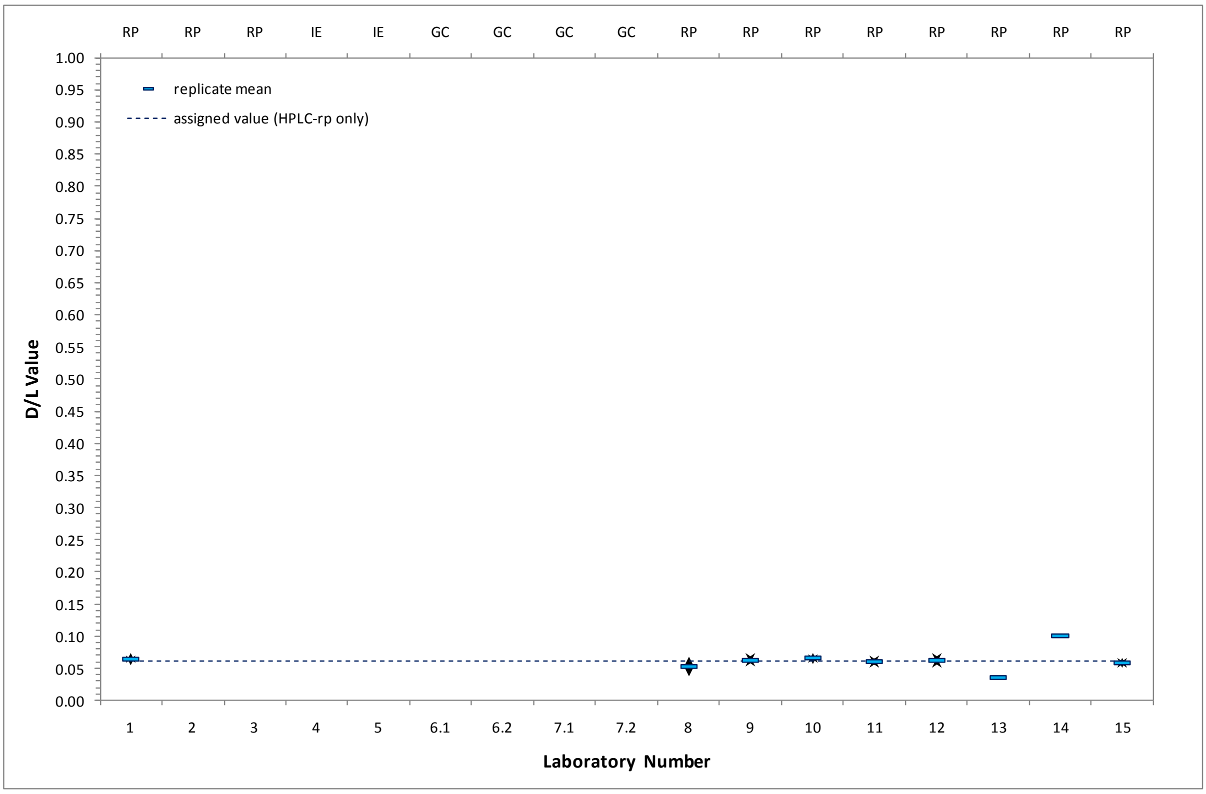


Figure 6.34: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Tyrosine** D/L Values in Ostrich Egg Shell (A) Test Material

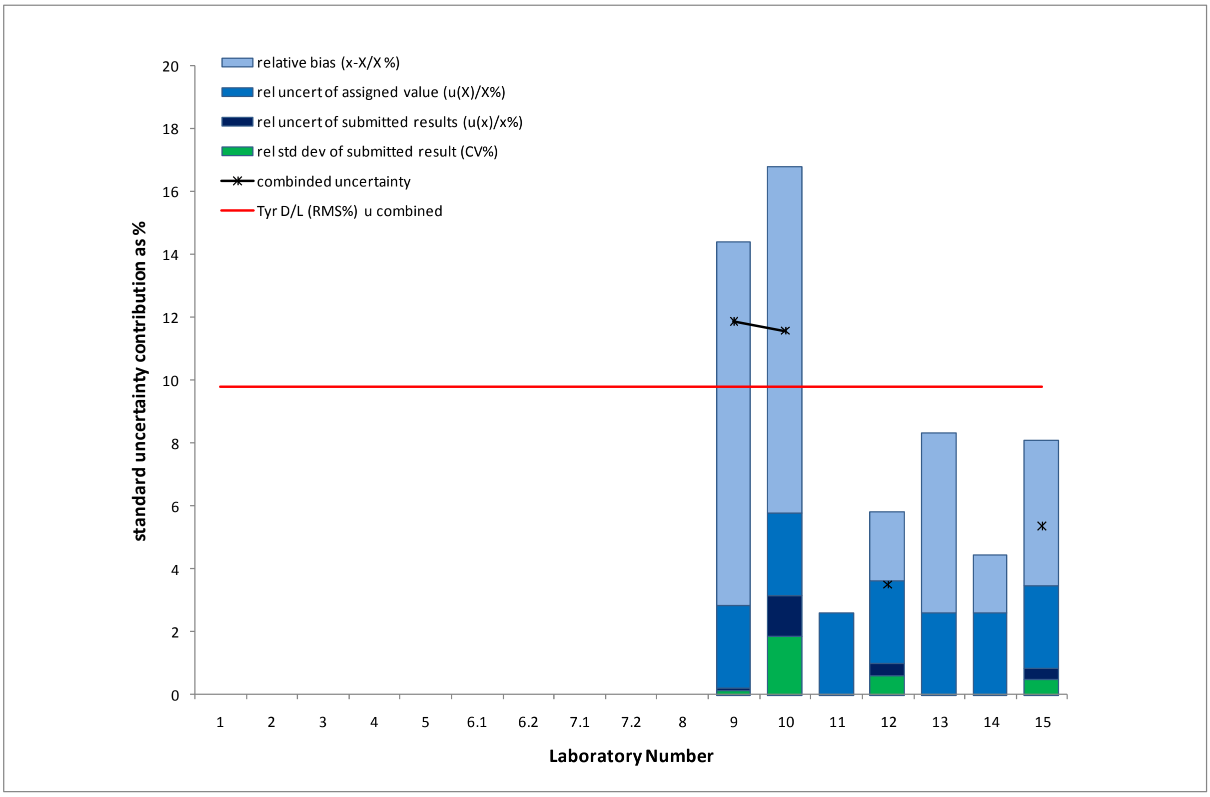
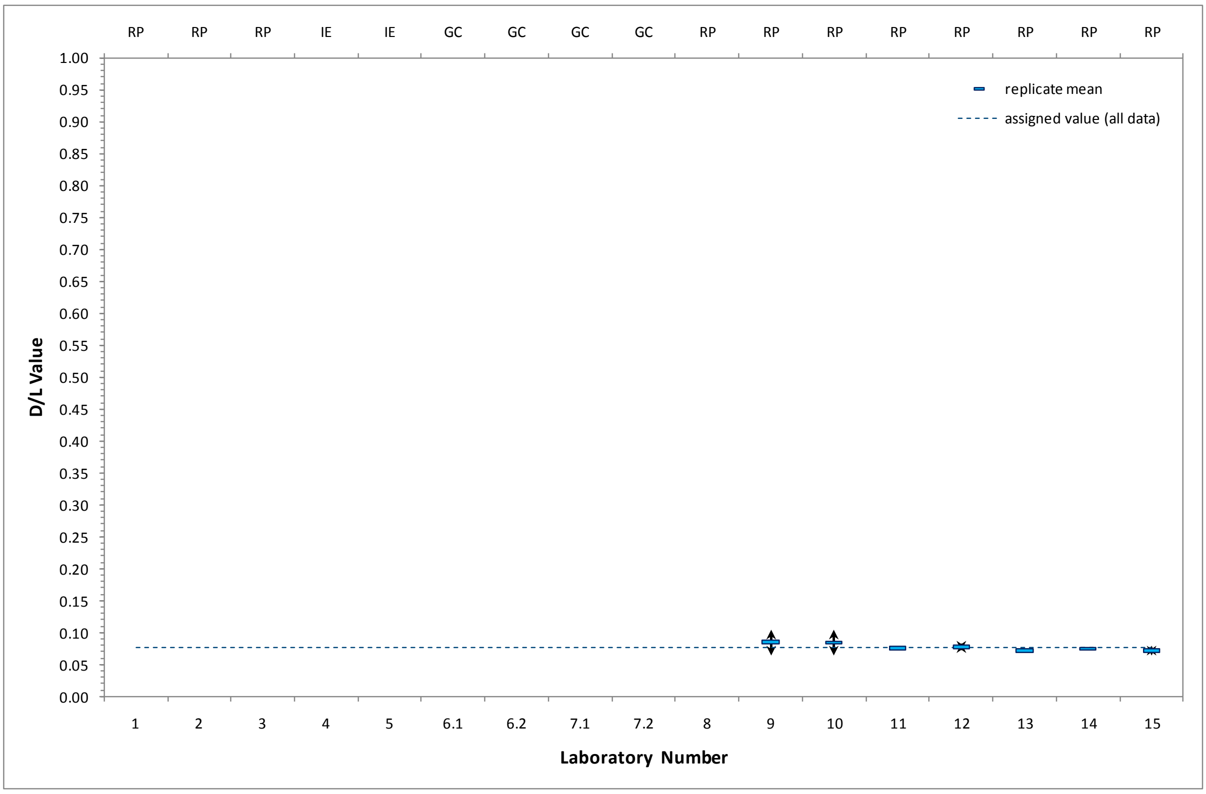


Figure 6.35: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Tyrosine** D/L Values in Ostrich Egg Shell (A) Test Material



Appendix 1: Analytical Methods Used by Participants

Reverse Phase HPLC/ HPLC-Ion Exchange

REFERENCES	
Please give details of any method relevant references;	
Kaufman & Manley 1998	009, 010, 011, 012, 013, 014, 015
HYDROLYSIS FOR THAA's	
Sample Weight used for analysis (mg):	
3.5 – 5 mg 1 – 10 mg >10 – 20 mg	003 008, 009, 010, 011, 012, 013, 014, 015 001, 002, 004, 005,
Vials used for hydrolysis:	
Glass	001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015
Acid Used:	
7M HCl	001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015
Vials flushed with N_2:	
Yes	001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015
Please give details of any other treatment prior to hydrolysis:	
Comments received; 1)20µl/mg of 7M HCl added to samples 2)2ml hydrolysis vials used 3)samples weighed & transferred to microvial or 4ml vial depending on size.	 001, 009, 010, 011, 012, 013, 014, 015 009, 010, 011, 012, 013, 014, 015 002, 003, 004, 005
Oven Temperature (°C):	
100 °C 110 °C	001 009, 010, 011, 012, 013, 014, 015
Heating Time (hours):	
6 hrs 20 hrs 22 hrs 24 hrs	002, 003 001 004, 005, 008 009, 010, 011, 012, 013, 014, 015
Was sample dried prior to analysis?:	
Yes	001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015
Please give details of sample drying conditions:	
Under vacuum Ambient / room temp Dried overnight	001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015 001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015 001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015

THAA's REHYDRATION	
Volume of rehydration fluid added as µl/mg of original sample	
10 µl/mg 20 µl/mg	001 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015
Internal Standard Used?:	
L-homo-Arginine Norleucine	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015 004, 005
Concentration of Internal std used (M):	
0.03 mM 0.01mM 6.25 mM	001 002, 003, 008, 009, 010, 011, 012, 013, 014, 015 004, 005
Source / supplier of internal standard:	
Sigma Sigma Aldrich (Fluka)	001, 002, 003, 004, 005 008
Other constituents and their concentrations (M or mM) in rehydration fluid:	
0.01M HCl 1.5mM Sodium Azide	002, 003, 004, 005, 009, 010, 011, 012, 013, 014, 015 009, 010, 011, 012, 013, 014, 015
ANALYSIS	
Please state method used	
Reverse phase HPLC Ion Exchange HPLC	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015 004, 005
Instrument used	
Agilent 1100 Series Agilent / Hewlet Packard 1100 Series Agilent 1200 Series Agilent 6890 GC, Flame Ionization	001, 008, 009, 012, 013 002, 003, 010, 011, 014, 015 004, 005 006, 007
Pre-column Derivatization Reagent constituents and their concentrations (M or mM):	
OPA 170 mM IBLC 260 mM Potassium borate buffer 1M	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015 001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015 001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
pH adjusted to:	
10.4	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
Sample injection volume (µl)	
2 µl 4 µl 20 µl	001, 002, 003, 009, 010, 011, 012, 013, 014, 015 008 004, 005

HPLC COLUMN	
Column Make/Type & Phase(i.e.; Hypersil BDS)/ Batch Number:	
Thermo/Hypersil BDS C18/0742018X	001
Hypersil BDS	009, 010, 011, 012, 013, 014, 015
Hypersil BDS /5/120/4772	002, 003
Pickering Labs Sodium Cation Exchange	004, 005
Supelcosil LC-18-DB(rp)/6520/5-1452	008
Column Packing:	
Silica	002, 003, 008
Sodium	004, 005
Functional group; C_{18}	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
End capped (Yes)	002, 003, 008
Column width (mm)	
3mm	001, 002, 003, 004, 005
5mm	009, 010, 011, 012, 013, 014, 015
Column length (mm)	
250mm	001, 002, 003, 004, 005, 009, 010, 011, 012, 013, 014, 015
Guard Column not used	
No	001, 002, 003, 004, 005
HPLC Column Temperature (°C):	
25 °C	001, 009, 010, 011, 012, 013, 014, 015
30 °C	002, 003, 004, 005, 008
MOBILE PHASE	
Mobile phase programme:	
Gradient	001, 002, 003, 004, 005, 009, 010, 011, 012, 013, 014, 015
Mobile phase components (please state; i.e.; sodium acetate buffer/ methanol/ acetonitrile):	
Sodium acetate Buffer (pH 6.00)	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
Methanol	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
Acetonitrile	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
Sodium citrate buffer (pH 3.12)	004. 005
Sodium citrate buffer (pH 3.86)	004, 005
Sodium chloride buffer (pH 11.5)	004, 005
Sodium acetate Buffer (pH 6.00) Gradient: Starting % | Final %| time (mins) | flow rate (ml/min)	
95%|76.6%|31mins|0.56ml/min	001a
76.6%|46.2%|95min|0.60ml/min	001b
95%|5%|83min|0.500ml/min	002, 003
95%|50%|88min|0.560ml/min	008
95%| %|95min|0.56ml/min	009, 010, 011, 012, 013, 014, 015

MOBILE PHASE continued	
Methanol Gradient: Starting % | Final %| time (mins) | flow rate (ml/min)	
5%|23%|31mins|0.56ml/min 23%|48.8%|95min|0.60ml/min 5%|95%|83min|0.500ml/min 5%|45%|88min|0.560ml/min 5%|50%|95min|0.56mi/min	001a 001b 002, 003 008 009, 010, 011, 012, 013, 014, 015
Acetonitrile Gradient: Starting % | Final %| time (mins) | flow rate (ml/min)	
0%|0.4%|31mins|0.56ml/min 0.4%|5%|95min|0.60ml/min 0.4%|5%|83min|0.500ml/min 0%|5%|88min|0.560ml/min 0%|5%|95min|0.56ml/min	001a 001b 002, 003 008 009, 010, 011, 012, 013, 014, 015
Sodium citrate buffer (pH3.12) Gradient: Starting % | Final %| time (mins) | flow rate (ml/min)	
100%|0%|99mins|0.140ml/min	004, 005
Sodium citrate buffer (pH3.86) Gradient: Starting % | Final %| time (mins) | flow rate (ml/min)	
0%|0%|99mins|0.140ml/min	004, 005
Sodium chloride buffer (pH11.5) Gradient: Starting % | Final %| time (mins) | flow rate (ml/min)	
0%|100%|99mins|0.140ml/min	004, 005
Post-column Derivatization Reagent constituents and their concentrations (M or mM):	
Boric Acid 0.5M OPA 0.0075M Ethanol 1% 2-mercapthoethanol 0.00075%	004,005 004,005 004,005 004,005
pH adjusted to 10.4	004,005
DETECTION	
Detector Type:	
Fluorescence	001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015
Excitation wavelength (nm):	
230 250 335 340	008, 009, 010, 011, 012, 013, 014, 015 002, 003 001 004, 005
Emission wavelength (nm):	
410 445 455	002, 003 001, 008, 009, 010, 011, 012, 013, 014, 015 004, 005

Gas Chromatography

REFERENCES	
Please give details of any method relevant references;	
Goodfriend 1991 with modifications	006, 007
HYDROLYSIS FOR THAA's	
Sample Weight used for analysis (mg):	
75 - 90 mg	006, 007
Vials used for hydrolysis:	
Glass	006, 007
Acid Used:	
6M HCl	006, 007
Vials flushed with N_2:	
Yes	006, 007
Please give details of any other treatment prior to hydrolysis:	
Comments received (006, 007); Samples weighed into hydrolysis vials without drying; fossil samples are always dried in vacuo prior to weighing for hydrolysis.	
Oven Temperature (^{o}C):	
105 ^{o}C	006, 007
Heating Time (hours):	
22 hrs	006, 007
SAMPLE CLEAN UP / DESALTING	
Was cation exchange resin used?	
No	006, 007
Was HF used to separate amino acids from precipitate?	
Yes	006, 007
Was sample dried prior to Derivatization?:	
Yes	006, 007
Please give details of sample drying conditions:	
Under nitrogen stream Drying Temp; 50 ^{o}C (in heating block) Drying time; 1 hr	006, 007 006, 007 006, 007

SAMPLE CLEAN UP / DESALTING continued	
Comments received (006, 007); After HF removal of Ca, solution of AA was dried under N2 to remove HF, then transferred with 1N HCl to a glass vial for additional N_2 drying and vacuum oven drying (total drying time ~2 hours at 60 deg C). This dried residue was then ready for esterification.	
ESTERIFICATION	
Esterification reagents:	
isopropanol	006, 007
Esterification conditions:	
Flushed under nitrogen Oven Temperature; 50°C Heating time; 1hr	006, 007 006, 007 006, 007
Was sample dried prior to acylation?:	
Yes	006, 007
Please give details of sample drying conditions:	
Under vacuum Under nitrogen stream Drying Temp; 55 °C Drying time; 1 hr	006, 007 006, 007 006, 007 006, 007
ACYLATION	
Acylation reagents:	
TFAA	006, 007
Acylation conditions:	
Flushed under nitrogen Room Temperature Heating time; 2hr minimum	006, 007 006, 007 006, 007
Comments received (006, 007); Isopropanol has to be removed before TFA can be added (with Methylene chloride)	
Was sample dried prior to GC analysis?	
Yes	006, 007
Please give details of sample drying conditions:	
Flushed under nitrogen Room Temperature Heating time; <5 minutes	006, 007 006, 007 006, 007
Comments received (006, 007); Derivative is in TFA/Meth Chloride – this solution was dried under N_2 and transferred to small vials for storage and GC injection; final solution containing derivative is in cyclohexane. Derivatives are injected on GC using cyclohexane	

THAA's REHYDRATION	
Volume of rehydration fluid added as µl	
20 – 30 µl	006, 007
Internal Standard Used?:	
No	006, 007
ANALYSIS	
Sample injection volume (µl)	
1 -3 µl	006, 007
GC injection mode:	
Splitless	006, 007
GC COLUMN	
Column Type;	
Capillary	006, 007
Column Make / Batch Number:	
Alltech, Catalog #13633, Serial # 5653, purchased in 1998, in continuous use	006, 007
Column Packing:	
Chiral Phase: Chirasil-val	006, 007
Column width (mm)	
0.25mm	006, 007
Column length (mm)	
25m	006, 007
Column Temperature (^{o}C):	
See below for program	006, 007
Mobile phase / Carrier gas	
Helium	006, 007
Mobile phase flow rate (ml/min):	
Flow variable with temperature; pressure 7.6psi	006, 007

DETECTION	
Detector Type:	
Flame ionisation	006, 007
Comments received (006, 007); NDP not used for these samples, but used in previous studies – both NPD and FID give same D/L values	

ANYTHING ELSE?

Please use this space for any additional information you would like to record concerning method details not covered above:

Comments received (006, 007);

Summary of the preparation sequence:

1) Dissolution in stoichiometric amount of conc. HCl to bringfinal solution to 6N

2) Purge with N2, seal hydrolysis tube, hydrolyse for 22 hoursat 105 deg.

3) After hydrolysis, HCl solution is transferred to plasticcentrifuge tube and appropriate amount of HF is added to remove Ca. After centrifuging, solution is transferred to another plastic tube for N2 drydown in a heating block (~60 deg). Drydown requires about one hour.

4) Dried residue is transferred using ~0.2 ml 1N HCl to a screwcap vial. This solution is dried with N2, then further dried in a vacuum oven (1 hour, 50 deg.) prior to esterification with isopropanol.

5) Isopropanol esterification – one hour at 105 deg.

6) Isopropanol is then dried down with N2 in 50 deg heating block (~10 minutes), then methylene chloride (Dichloromethane, or DCM) and TFA are added. This complete derivative is then usually stored overnight prior toGC analysis.

7) The DCM/TFA solution is transferred to a small GC vial, dried with N2, then cyclohexane is added to ready the derivative for GC injection. The amount of cyclohexane is variable depending on the sample size, but there is no “formula” for this because the GC analysis is not quantitative. Derivatives remain in the cyclohexane solution until GC injection – in most cases, five or six chromatograms are obtained over a period of one to two weeks. Injection amounts are usually 1 ul; if samples are

small, 2 or even 3 ul will be injected.

8) GC temperature program: inject at 60 deg, hold for one minute; 20 deg/min up to 80 deg; hold for 10 minutes; 0.85 deg/min to 135 deg, 1 minute hold; 5 deg/min to 160, 10 minutes hold; recycle. All important peaks are eluted within 100 minutes; last phases of temperature program are to clean out the column.

Internal Quality Control

INSTRUMENT CALIBRATION	
Was the instrument calibrated prior to analysis?	
Yes, prior to analytical run Yes, within the last year No	001 008 002, 003, 004, 005, 006, 007, 009, 010, 011, 012, 013, 014, 015
If Yes, type of calibration:	
Calibration curve/std addition-single level Calibrated by Agilent Technician	001 008
If Yes, what reference materials / standards are used?	
In-house std solution(s) NB: Solution prepared from single powdered AA standards	001
Source of reference materials/standards:	
Sigma	001
RECOVERY OR INTERNAL STANDARD	
Was % recovery determined?	
No	001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015
If No, was an internal standard used?	
Yes, as component of rehydration fluid	001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015
Internal Standard Used?:	
L-homo-Arginine Norleucine No	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015 004, 005 006, 007
Concentration of Internal std used (M):	
0.03 mM 0.01mM 6.25 mM	001 002, 003, 008, 009, 010, 011, 012, 013, 014, 015 004, 005
Source / supplier of internal standard:	
Sigma Sigma Aldrich (Fluka)	001, 002, 003, 004, 005 008

D/L RATIO CALCULATION	
Do you routinely calculate concentrations?	
Yes No	001, 009, 010, 011, 012, 013, 014, 015 002, 003, 004, 005, 006, 007, 008
Comments received; (001) Concentration of a single enantiomer in solution (milimol/L)= (enenatiomer area x Internal Standard concentration)/ Internal Standard area Concentration of a single enantiomer in the sample (picomol/mg)= [Concentration of enantiomer in solution (milimol/L) x Volume of rehydration fluid added (L) x 10-9 picomol/milimol)]/sample weight (mg) (006, 007): Only peak areas are reported under most circumstances but both are measured to check for reliability and peak distortion/overload.	
D/L values are routinely calculated using:	
Peak heights Peak areas Concentrations based on peak areas	004, 005, 006, 007 001, 002, 003, 006, 007, 008 009, 010, 011, 012, 013, 014, 015
QUALITY CONTROL	
Do you routinely use lab QC materials or standards.	
Yes	001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015
If Yes,are they:	
In-house std solution(s) (Matrix-matched) ILC stds (Wehmiller)	001, 002, 003, 004, 005, 009, 010, 011, 012, 013, 014, 015 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015
Source of QC materials:	
Sigma J.F.Wehmiller	001, 002, 003, 004, 005, 009, 010, 011, 012, 013, 014, 015 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015
How do you use QC materials?	
Control charts Visual inspection of chromatograms/data D/L comparison to lit Comparison in ILC's with long term mean	001, 002, 003, 004, 005 008, 009, 010, 011, 012, 013, 014, 015 008 006, 007
MEASUREMENT UNCERTAINTY	
How do you determine Measurement Uncertainty (MU) of your data	
As the standard deviation	001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015
If you do, how often do you determine the MU?	
Routinely per run Approx once a month When its needed As the SD of multiple chromatograms from each derivative.	008 002, 003, 004, 005, 001, 009, 010, 011, 012, 013, 014, 015 006, 007, 009, 010, 011, 012, 013, 014, 015

Appendix 2: Glossary of Abbreviations, Symbols, Terms & Definitions

Abbreviations

ANOVA	Analysis of Variance
CRM	Certified Reference Material
CV	Coefficient of Variation
EQC	External Quality Control
IQC	Internal Quality Control
MU	Uncertainty of Measurement / Measurement Uncertainty
PT	Proficiency test
QA	Quality Assurance
QC	Quality Control

Symbols

k	Coverage Factor
RMS_{bias}	Bias Root Mean Square
$RSD_L\%$	Relative Between Sample Standard Deviation (expressed as a percentage)
$RSU\%$	Relative Standard Uncertainty (expressed as a percentage)
$RSD\%$	Relative standard deviation (expressed as a percentage)
$RSD_r\%$	Relative Repeatability standard deviation (expressed as a percentage)
$RSD_R\%$	Relative Reproducibility standard deviation (expressed as a percentage)
s_{an}	(Homogeneity) Analytical Precision
s_{an}^2	(Homogeneity) Analytical Variance
s_{sam}	(Homogeneity) Sampling Precision
s_{sam}^2	(Homogeneity) Sampling Variance
s_{all}^2	(Homogeneity) Total Permissible Sampling Variance
s, sd or σ	Standard Deviation
S_L	Between-sample standard deviation
S_r	Repeatability Standard Deviation
S_R	Reproducibility Standard Deviation (Inter-Laboratory)
S_{RW}	Reproducibility Standard Deviation (Intra-Laboratory) or Intermediate Precision
σ_p	Target Standard Deviation
σ_h	Homogeneity Target standard deviation
$\hat{\sigma}$	Assigned Value standard deviation
$u(x)$	Standard Uncertainty

$u(\hat{X})$	Standard Uncertainty of the Assigned Value
$u(bias)$	Standard Uncertainty due to Bias
$u(\bar{x})$	Standard Uncertainty of Participant's Results
u_c	Combined (standard) Uncertainty
U	Expanded Uncertainty
x or x_i	Submitted Result or Value
$\bar{x}$	Measurement Result / Mean submitted result
$\hat{X}$	Assigned Value

Terms and Definitions

Specific references for terms that can be found in International Standards or guidance documents have been given in brackets at the end of each definition. Here, **VIM** refers to '*International vocabulary of metrology*' (JCGM 200:, 2008), **GUM** refers to the '*Guide to the expression of uncertainty in Measurement'* (JCGM 100:, 2008) and **ISO (1)**,refers to (ISO 5725-1, 1994) on the '*Accuracy (trueness and precision) of measurement methods and results'*. Terms shown in bold indicate further definitions that may be found in this section.

Readers are recommended to consult these documents for additional notes and comments not included here.

Accuracy

closeness of agreement between a measured result and the true value (if it could be known), or a reference value. (VIM 2.13)

> NOTE 1; Accuracy is a concept that cannot be directly quantified. It does not possess a numerical value.
>
> NOTE 2; Accuracy describes **random** and **systematic error** effects and as such is composed of both **precision** and **bias** components.

Analysis of Variance (ANOVA)

A group of statistical techniques that enable the different contributions from various sources of the observed variance in experimental data to be separated and estimated. (Currell and Dowman, 2005, Miller and Miller, 2005).

> NOTE 1; A one-way ANOVA uses the F-test to compare the effect of one factor plus the experimental precision, eg; the effect of the measurement process on different samples, (between-sample variance) against the inherent experimental precision (within-sample variance).
>
> NOTE 2; Whilst it is possible to carry out the analysis by hand more commonly statsistical software packages are more convenient such as the Excel Data Analysis tools as this also carries out the F-test evaluation at the same time.

Assigned Value $\hat{X}$

The best estimate of the true value of the measurand.

> NOTE; This may be the certified reference value of a CRM, a reference value from a reference laboratory or the consensus value from participants' results calculated as the robust mean, median or mode.

Assigned Value standard deviation ($\hat{\sigma}$)
Standard deviation of the assigned value.

NOTE; This may be the robust standard deviation, sMAD (median absolute deviation) or SEM (standard error of the mode)

Between-sample standard deviation (S_L);
The precision or dispersion between independent measurements carried out on different samples of the same material under **reproducibility conditions.**

NOTE: it includes the between-operator, between-day, between-instruments, and between-laboratory variability's, etc. and is a component of **reproducibility standard deviation**. It is determined using **ANOVA**, such that;

$$s_L = \sqrt{\frac{between\ group\ mean\ square - within\ group\ mean\ square}{n}}$$

Bias
estimate of a systematic measurement error (VIM 2.18)

$$bias = (\bar{x} - \hat{X})$$

Bias Root Mean Square (RMS_{bias})
A component of the bias standard uncertainty taking into account both the bias and bias variation. See **Standard uncertainty due to bias ($u(bias)$).**

Certified Reference Material (CRM);
a reference material accompanied by certified traceable measurement and uncertainty values determined using validated procedures (VIM 5.14)

Cochran's Test
A statistical test that detects extreme variances between observations by calculating the Cochran's (C) value as the ratio between the largest squared difference (D^2_{max}) to the sum of all the squared differences ($\sum D_i^2$) and comparing this against tabulated critical values. (ISO 5752-2: 1994)

$$C = D^2_{max} \Big/ \sum D_i^2$$

Coefficient of Variation ($CV\%$) (expressed as a percentage).
See **Relative standard deviation ($RSD\%$)**

Combined (standard) Uncertainty (u_c)
The combined standard uncertainty of a measurement result taking into account various contributions from different standard uncertainty sources. (GUM 2.3.4)

NOTE 1; There are two common rules for the combination of **standard uncertainty** values which depend on the model used for deriving the measurement value;

Eg; a). If the model involves the addition or subtraction of values,
i.e.; $y = (a + b + c \ldots)$then the combined standard uncertainty, $u_c(y)$ is given by;

$$u_c(y(a, b, c \ldots)) = \sqrt{u(a)^2 + u(b)^2 + u(c)^2 + \ldots}$$

Eg; b). If the model involves the product or quotient of values,
i.e.; $y = (a \times b \times c \ldots)$ or $y = a/(b \times c \ldots)$ then the combined standard uncertainty, $u_c(y)$ is given by;

$$u_c(y(a, b, c \ldots)) = y\sqrt{\left(\frac{u(a)}{a}\right)^2 + \left(\frac{u(b)}{b}\right)^2 + \left(\frac{u(c)}{c}\right)^2 + \ldots}$$

NOTE 2; For proficiency testing the format given in the first example has been used, thus;

$$u_C = \sqrt{S_{Rw}^2 + u(\bar{x})^2 + u(\hat{X})^2 + (bias)^2}$$

Where; $\sqrt{S_{Rw}^2}$ = uncertainty due to precision, and
$\sqrt{u(\bar{x})^2 + u(\hat{X})^2 + (bias)^2} = u(bias)$ i.e.; the **uncertainty due to bias.**

Coverage Factor (k)

Factor used to multiply the combined uncertainty by in order to derive the Expanded uncertainty value.

NOTE; For large data sets where the distribution approximates to normality the value of k to use is taken from the level of confidence required in the measurement result. Most often a 95% or 2 standard deviation level of confidence is required for the reporting of measurement results, thus *k=2*.

For smaller data sets where the distribution of measurement results is better described by a t-distribution, the equivalent t-value is used as the multiplier, thus $k=t_{(0.5,df)}$.

Error

measured quantity value minus a reference value or true value (VIM 2.16)

NOTE 1; To some extent the concept of error is a theoretical one as it is not possible to be sure of a measurand's true value, only a best estimation of it from measurement determinations. If a reference value is to be used then it is more accurate to determine the precision and bias as estimates of random and systematic error contributions which can be quantified.

Expanded Uncertainty (U)

A quantity defined by a specified interval (i.e.; 2 standard deviations) or confidence level (i.e.; 95% confidence) about the measurement result and describes the dispersion where a large number of repeated **measurement results** would be expected to lie.

$U = u_c \times k$ where k = the **coverage factor**, and
u_c= the **combined uncertainty**

Experimental standard deviation of the mean.

See **Standard Uncertainty ($u(x)$)**

External Quality Control (EQC)

See **Quality Control (QC).**

F_1 and F_2

Are constants used to test the hypothesis that there is no significant evidence that the sampling standard deviation exceeds the allowable fraction of the target standard deviation and that the test for sufficient homogeneity has been passed (Fearn, T. and Thompson, M., 2001).

$$s_{sam}^2 = F_1 s_{all}^2 + F_2 s_{an}^2$$

Values for F_1 and F_2 may be derived from statistical tables;

$$F_1 = \frac{\chi^2_{(m-1,0.95)}}{m-1}$$ where m = the number of samples measured in duplicate

$$F_2 = \frac{F_{(m-1,m,0.95)}-1}{2}$$

NOTE; The (Fisher) F-Test is a test for significant differences between the variances of two data sets and compares random error effects. The F-test may also be used within other tests such as ANOVA, (Currell, G., & Dowman, A.,2005, Miller, J.N, & Miller, J.C., 2005)

Thus; F-statistic $F = {s_a^2}/{s_b^2} \; or \; = {MS_{between}}/{MS_{within}}$

(Homogeneity) Analytical Precision (s_{an})

The homogeneity within-sample standard deviation for the replicate values (i.e.; a and b) used in the test for sufficient homogeneity of the test materials. Calculated from the ANOVA within group mean square;

$$s_{an} = \sqrt{MS_w}$$

(Homogeneity) Analytical Variance (s_{an}^2)

The square of the analytical precision. . Calculated from the ANOVA within group mean square;

$$s_{an}^2 = MS_w$$

(Homogeneity) Sampling Precision (s_{sam})

The homogeneity between-sample standard deviation for the samples (i.e.; 1, 2…10) used in the test for sufficient homogeneity of the test materials. Calculated from the ANOVA between and within group mean square values;

$$s_{sam} = \sqrt{\frac{MS_b - MS_w}{2}}$$

(Homogeneity) Sampling Variance (s_{sam}^2)

The square of the sampling precision. Calculated from the ANOVA between and within group mean square values;

$$s_{sam}^2 = \frac{MS_b - MS_w}{2}$$

Homogeneity Target standard deviation (σ_h).

In the absence of an external value for target standard deviation (σ_p), a target value sufficient homogeneity (σ_h)can be determined using fitness-for-purpose criteria.

(Homogeneity) Total Permissible Sampling Variance (s_{all}^2)

The total allowable between-sample variance that must not be exceeded by the sampling variance in order for the test materials to be considered homogeneous. $\boldsymbol{s_{all}^2}$ is derived from the homogeneity target standard deviation (either $\sigma_p \; or \; \sigma_h$).

$$s_{all}^2 = (0.3 \times \sigma_p)^2$$

Intermediate conditions

Independent measurement results obtained for identical test items using the same measurement procedure under a specified set of conditions within the same laboratory that include, different operators, different operating conditions, different locations over any given period of time, (VIM 2.22). See **Reproducibility Standard Deviation (Intra-Laboratory) or Intermediate Precision (S_{RW})**

Internal Quality Control (IQC)

See **Quality Control (QC)**

Measurement Result / Mean submitted result ($\overline{x}$)

The average of an individual participant's replicate measurement results for the same analyte in the proficiency test.

Precision
closeness of agreement between repeated measurement results on the same material under specified conditions (VIM 2.15)

> NOTE 1; Precision can be quantified and usually expressed as a measure of imprecision such as standard deviation, variance, relative std dev or CV and is a measure of random error.
>
> NOTE 2; Specific measurement conditions can be repeatability, intermediate or reproducibility conditions.

Proficiency test (PT);
An **external quality control (EQC)** procedure through which the **accuracy** of a laboratory's measurement result can be objectively evaluated. Performance is assessed by providing a comparison of **trueness** with other participating laboratories

> NOTE: **Trueness** is determined through the evaluation of laboratory **bias** against a reference value. This may be presented as **z-scores** or other assessment of **bias**.

Quality Assurance (QA);
Documented procedures that describe a quality management system designed to control activities and maintain a quality output.

Quality Control (QC);
Specific activities that are carried out in order to implement the procedures documented under the **Quality Assurance** programme.

> NOTE; This may be in the form of **Internal Quality control (IQC)** that are carried out internally by the organization such as method validation, calibration, control charts, etc, or **External Quality Control (EQC)** coordinated by an external organization such as interlaboratory comparisons eg; proficiency tests or collaborative trails.

Random error
component of measurement error that in replicate measurements varies unpredictably (VIM 2.19)

> NOTE 1; A random error value is determined as the precision that would result from a number of replicate measurements of the same measurand, expressed as a distribution.

Relative Bias % (expressed as a percentage)
Bias divided by the assigned value (x 100)

$$relative\ bias\ \% = \frac{(\bar{x} - \hat{X})}{\hat{X}} \text{ x } 100$$

Relative Between Sample Standard Deviation ($RSD_L\%$), (expressed as a percentage)
The **between-sample standard deviation** divided by the (average) measurement result (x 100)

$$RSD_L\% = \left({}^{S_L}/_{\bar{x}}\right) \times 100$$

Relative Standard Uncertainty ($RSU\%$), (expressed as a percentage)
The **standard uncertainty** divided by the (average) measurement result (x 100)

$$RSU\% = \left({}^{u(\bar{x})}/_{\bar{x}}\right) \times 100$$

Relative standard deviation ($RSD\%$) or Coefficient of Variation ($CV\%$) (expressed as a percentage)
The **standard deviation** divided by the (average) measurement result (x 100)

$$RSD\%\ or\ CV\% = \left({}^{S}/_{\bar{x}}\right) \times 100$$

Relative Repeatability standard deviation ($RSD_r\%$), (expressed as a percentage)
The **repeatability standard deviation** divided by the (average) measurement result (x 100)

$$RSD_r\% = \left({}^{s_r}/_{\bar{x}}\right) \times 100$$

Relative Reproducibility standard deviation ($RSD_R\%$), expressed as a percentage
The **Reproducibility standard deviation** divided by the (average) measurement result (x 100)

$$RSD_R\% = \left({}^{s_R}/_{\bar{x}}\right) \times 100$$

Repeatability conditions ;
Independent measurement results are obtained for identical test items under a specified set of conditions that include the same measurement procedure, same measurement system or laboratory, same operators, same operating conditions, same location and in as short a time as period as possible, (VIM 2.20, ISO (1) 3.14). See **Repeatability Standard Deviation (S_r)**

Repeatability Standard Deviation (S_r)
The dispersion or precision of replicate measurement values carried out under repeatability conditions (ISO (1) 3.15)

NOTE; Often calculated using **ANOVA** from the within group mean square (MS), such that;

$$s_r = \sqrt{within\ group\ mean\ square}$$

Eg; a).Within-sample (or instrumental/analytical) repeatability standard deviation is the dispersion of replicate instrumental measurements carried out on the same sample in the same analytical run, eg; an individual laboratory's replicate PT results.

b). Intra-laboratory (or method + analytical) repeatability standard deviation is the dispersion of independent measurements carried out by a single laboratory on different samples of the same material, under repeatability conditions, eg. From Intra-laboratory method validation data or homogeneity analytical precision data (s_{an}).

c). Inter-laboratory repeatability (laboratory+method+analytical) standard deviation is the dispersion of independent measurements carried out by more than one laboratory on different samples of the same material, under repeatability conditions, eg, collaborative trial precision data.

Reproducibility Conditions;
Independent measurement results obtained for identical test items using the same measurement procedure under a specified set of conditions that include, different measurement systems and laboratories, different operators, different operating conditions, different locations over any given period of time, (VIM 2.24, ISO (1) 3.18). See **Reproducibility Standard Deviation (Inter-Laboratory) (S_R)**

Reproducibility Standard Deviation (Inter-Laboratory) (S_R)
The overall dispersion or precision of independent measurement values carried out on different samples of the same material by different laboratories, under **reproducibility conditions** and incorporates both within (repeatability) and between-sample precision estimates (ISO (1) 3.19)

Thus; $$s_R = \sqrt{s_r^2 + s_L^2}$$

Eg; a). The Inter-laboratory reproducibility standard deviation (S_R) obtained from a collaborative trial represents the maximum dispersion for the measurement procedure carried out across laboratories and provides an estimate of best practice for the measurement procedure for a specified matrix / analyte/ concentration. Providing a laboratory's own repeatability is in agreement with the inter-laboratory repeatability precision estimate, then the laboratory can claim the Reproducibility standard deviation from a collaborative trial as their own **standard uncertainty** estimate.

Reproducibility Standard Deviation (Intra-Laboratory) or Intermediate Precision (S_{RW})

The overall dispersion or precision of independent measurement values carried out on different samples of the same material by the same laboratory, under **reproducibility conditions** and incorporates both within (repeatability) and between-sample precision estimates (VIM 2.23)

Thus; $s_{RW} = \sqrt{s_r^2 + s_L^2}$

Eg; Intra-laboratory reproducibility standard deviation (S_{RW}) represents the maximum dispersion for the measurement procedure carried out by an individual laboratory and is often used in method validation as the method precision for a particular matrix / analyte /concentration and used as the **standard uncertainty**.

Standard Deviation (s, sd or σ)

A term used to describe the dispersion or spread of measurement values and has the same units as the measurement value.

NOTE; by convention the symbol used for standard deviation depends on whether it is describing sample statistics or population parameters. Thus;

Sample statistics; $s = \sigma_{n-1} = \sqrt{\frac{\sum_1^n (x_i - \bar{x})^2}{n-1}}$

Population parameters; $\sigma = \sqrt{\frac{\sum_1^n (x_i - \mu)^2}{n}}$

Where x_i = individual measurement values
$\bar{x}$ = average measurement value for the sample
μ = population mean
n = number of measurement values or population size

Standard Error of the Mean.

See **Standard Uncertainty ($u(x)$)**

Standard Uncertainty ($u(x)$)

The uncertainty of a measurement result expressed as a standard deviation, (GUM 2.3.1)

NOTE; When determined from a series of repeated measurements this can also be found referred to in texts as the experimental standard deviation or standard error of the mean.

Thus; $u(x) = {}^{s}/_{\sqrt{n}}$

Standard Uncertainty of the Assigned Value ($u(\hat{X})$)

The uncertainty of the **Assigned Value,** expressed as a standard deviation, (GUM 2.3.1).

$u(\hat{X}) = {}^{\hat{\sigma}}/_{\sqrt{m}}$ where $\hat{\sigma}$ = the **assigned value** std dev
and m = the number of participants' measurement results

NOTE; $u(\hat{X})$ is also a component of the **standard uncertainty due to bias $u(bias)$**.

Standard Uncertainty due to Bias ($u(bias)$).

The uncertainty of the bias component of a participant's measurement result, expressed as a standard deviation, (GUM 2.3.1).

NOTE 1; An individual laboratory's standard uncertainty due to bias for a single proficiency test, is given as;

$u(bias) = \sqrt{(bias)^2 + u(\bar{x})^2 + u(\hat{X})^2}$

NOTE 2; An individual laboratory's standard uncertainty due to bias over multiple proficiency tests, is given as;

$$u(bias) = \sqrt{RMS_{bias}{}^{2} + u(\hat{X})^{2}}$$

where; RMS_{bias} = the **bias root mean square** and given as;

$$RMS_{bias} = \sqrt{\frac{\sum(bias_i)^2}{m}}$$

and $u(\hat{X})$= the average standard uncertainty of the assigned value;

$$u(\hat{X}) = \sum \hat{\sigma}_i \Big/ \sqrt{\sum n_i}$$

m = the number of proficiency tests or number of bias values, and
n = the number of participants' measurement results in each PT.

NOTE 3; It often helps to carry out these calculations as the relative percentage values.

Standard Uncertainty of Participant's Results ($u(\bar{x})$)

The uncertainty of a participant's submitted replicate results, expressed as a standard deviation, (GUM 2.3.1).

$$u(\bar{x}) = s_{\bar{x}} \big/ \sqrt{n}$$

where $s_{\bar{x}}$ = the std dev of replicate values
and n = the number of replicate values submitted

NOTE; $u(\bar{x})$ is also a component of the **standard uncertainty due to bias $u(bias)$**.

Submitted Result or Value (x or x_i)

An individual participant's submitted measurement result for the proficiency test.

Systematic Error

component of measurement error that in replicate measurements remains constant or varies predictably (VIM 2.17)

NOTE 1; A systematic error value is determined as the bias, i.e.; the difference between a measured result and the true or reference value. Measurement results should always be corrected where significant bias is detected.

Target Standard Deviation (σ_p)

The target value for standard deviation for the proficiency test used to calculate z-scores and assess homogeneity data.

NOTE; often determined independently from data external to the proficiency test, such as the reproducibility standard deviation (RSD_R%) from a collaborative trail or using a predictive model such as the Horwitz function when appropriate of fitness-for purpose criteria. The target std dev is usually matrix / analyte specific.

Eg; a) From a collaborative trial; $\sigma_p = \frac{RSD_R}{100} \times c$

where RSD_R = Relative Standard Deviation of Reproducibility from collaborative trial data, expressed as %

and c = concentration, i.e. the assigned value, $\hat{X}$, expressed in relevant units.

Eg; b) Using the Horwitz equation; $\sigma_p = 0.02c^{0.8495}$
Or modified form; for concentrations less than 120ppb (1.2×10^{-7}); $\sigma_p = 0.22c$
and for concentrations greater than 13.8% (0.138); $\sigma_p = 0.01c^{0.5}$

Where the concentration (*c)* is expressed as a mass fraction as shown in () above.

Trueness

closeness of agreement between the average of a large number of replicate measurement results and the true value (if it could be known) or a reference value (VIM 2.14)

NOTE 1; Trueness is a concept that cannot be directly quantified. It does not possess a numerical value.

NOTE 2; Trueness is usually expressed as bias and a measure of systematic error.

t-value

2-tailed t-value is used as a correction factor in the determination of confidence intervals for small values of n. Derived from the t-distribution for sample data sets and described using $t(\bar{x}, s)$, compared to the normal distribution for populations described as $N(\mu, \sigma)$. Values for t may be obtained from statistical tables. (Currell and Dowman, 2005, Miller and Miller, 2005).

Such that, for a 95% confidence interval; $CI = \bar{x} \pm \left[t_{(2,0.05,df)} \times \frac{\sigma}{\sqrt{n}}\right]$

NOTE; The (student's) t-Test is a test for significant differences between the mean of two data sets and compares systematic error effects.

Thus; t-statistic $t = {(x-\mu)}\Big/{s/\sqrt{n}}$

Uncertainty of Measurement / Measurement Uncertainty (MU)

A parameter associated with a measurement result (taken as the best estimate of the true value) and characterizes the dispersion of values that could be attributed to the measurement result, taking into account both random and systematic error contributions from all possible sources and represents the degree of doubt associated with the measurement result (GUM 2.2).

Welch-Satterthwaite formula

Formula used for deriving the effective degrees of freedom for the calculation of Expanded uncertainty, when various standard uncertainties are combined with differing degrees of freedom.

$$v_{eff} = u_c^4(y) \Big/ \sum \frac{u_i^4(y)}{v_i}$$

Where v_{eff} = the effective degrees of freedom,
v_i = degrees of freedom of individual uncertainty components,
u_c = combined standard uncertainty
u_i = individual uncertainty components.

z-Score

A standardized measure of laboratory bias derived from the assigned value and target standard deviation, enabling a comparison of performance between laboratories. Satisfactory performance is considered if a |z|≤2.

$$z = \frac{(x - \hat{X})}{\sigma_p}$$

Appendix 3: Tables of Critical Values

Student t-distribution

df	95%	99%	df	95%	99%
1	12.7100	63.6600	26	2.0555	2.7787
2	4.3027	9.9250	27	2.0518	2.7707
3	3.1824	5.8408	28	2.0484	2.7633
4	2.7765	4.6041	29	2.0452	2.7564
5	2.5706	4.0321	30	2.0423	2.7500
6	2.4469	3.7074	31	2.0395	2.7440
7	2.3646	3.4995	32	2.0369	2.7385
8	2.3060	3.3554	33	2.0345	2.7333
9	2.2622	3.2498	34	2.0322	2.7284
10	2.2281	3.1693	35	2.0301	2.7238
11	2.2010	3.1058	36	2.0281	2.7195
12	2.1788	3.0545	37	2.0262	2.7154
13	2.1604	3.0123	38	2.0244	2.7116
14	2.1448	2.9768	39	2.0227	2.7079
15	2.1315	2.9467	40	2.0211	2.7045
16	2.1199	2.9208	41	2.0195	2.7012
17	2.1098	2.8982	42	2.0181	2.6981
18	2.1009	2.8784	43	2.0167	2.6951
19	2.0930	2.8609	44	2.0154	2.6923
20	2.0860	2.8453	45	2.0141	2.6896
21	2.0796	2.8314	46	2.0129	2.6870
22	2.0739	2.8188	47	2.0117	2.6846
23	2.0687	2.8073	48	2.0106	2.6822
24	2.0639	2.7970	49	2.0096	2.6800
25	2.0595	2.7874	50	2.0086	2.6778

Factors F_1 and F_2 (95% significance level)

m	20	19	18	17	16	15	14	13	12	11	10	9	8	7
F_1	1.59	1.60	1.62	1.64	1.67	1.69	1.72	1.75	1.79	1.83	1.88	1.94	2.01	2.10
F_2	0.57	0.59	0.62	0.64	0.68	0.71	0.75	0.80	0.86	0.93	1.01	1.11	1.25	1.43

(Fearn and Thompson, 2001)

Cochran's Critical values (95% significance level)

No of Samples (m)	No of sample replicates (n) 2	3
2	99.9	97.5
3	96.7	87.1
4	90.7	76.8
5	84.1	68.4
6	78.1	61.6
7	72.7	56.1
8	68.0	51.6
9	63.9	47.8
10	60.2	44.5
11	57	41.7
12	54.1	39.2
13	51.5	37.1
14	49.2	35.2
15	47.1	33.5
16	45.2	31.9
17	43.4	30.5
18	41.8	29.3
19	40.3	28.1
20	38.9	27.1

(ISO 5725-2, 1994)

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Contact Details;

Please direct communications regarding this Report to;

Jo Powell

BioArCh, Dept of Archaeology,
University of York,
Biology S Block,
Wentworth Way,
YORK YO10 5DD. UK

tel; +44(0)1904 328806
email: jp588@york.ac.uk