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Amino Acid Racemization Proficiency Study

Report VI: MOLLUSC SHELL (B)

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1 INTRODUCTION

1.1 Amino Acid Racemisation

Amino Acid racemization (or epimerizationⁱ 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).

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 trial, 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 EPTISⁱⁱ 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

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

Mollusc Shell (B)

2.1 Preparation

Raw material for the mollusc shell test materials was generously provided by The University of Delaware, USA. Bulk material was prepared from the Pleistocene bivalve *Saxidomus*, previously referenced as ILC-A in Wehmiller's inter-laboratory comparison studies (Wehmiller, 1984; Wehmiller, 2012 (submitted)). The milled calcite/aragonite bulk mollusc shell powder was further ground using a sterile pestle and mortar and sieved, to $\leq 250 \mu\text{m}$ before finally being tumble-blended overnight on a roller mixer.

Half the finely powdered mollusc shell was then weighed (20 mg sub-samples) into sterile glass vials and labelled as Mollusc Shell (B). The remaining half of the powdered material was bleached for 48 hours using 50 μl of 12% NaOCl per mg of powder. After washing and drying this material was also measured and individual 20 mg sub-samples were weighed into sterile glass vials and labeled as Mollusc Shell (A). 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

Mollusc Shell (B) 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);

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

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 Mollusc Shell (B) 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 were then randomized and analysed as a single batch under repeatability conditions using reverse-phase HPLC.

The D/L results for the twenty 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. Sample 8 was identified as a Cochran's outlier in the evaluation of Glx, Ala and Phe data, and thus data for 8a and 8b were removed from the statistical evaluation of these particular amino acids. The D/L results and statistical evaluation are given in Table 3.1. Figure 3.2 shows the paired D/L values for each amino acid

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 Mollusc Shell (B) Test Material

sample id	analyte											
	Asx D/L		Glx D/L		Ser D/L		Arg D/L		Ala D/L			
	replicate 1	replicate 2										
1	0.419	0.408	0.223	0.216	0.477	0.434	0.779	0.820	0.387	0.331		
2	0.415	0.412	0.222	0.219	0.463	0.440	0.758	0.805	0.380	0.385		
3	0.414	0.402	0.220	0.210	0.434	0.355	0.857	0.734	0.392	0.358		
4	0.410	0.408	0.206	0.217	0.431	0.431	0.845	0.874	0.388	0.367		
5	0.406	0.405	0.219	0.216	0.435	0.433	0.695	0.866	0.400	0.368		
6	0.404	0.413	0.219	0.219	0.442	0.446	0.757	0.783	0.376	0.374		
7	0.402	0.412	0.206	0.217	0.351	0.458	0.742	0.687	0.355	0.367		
8	0.386	0.411	0.194	0.099	C	0.311	0.405	0.833	0.873	0.306	0.404	C
9	0.409	0.405	0.218	0.215	0.428	0.386	0.697	0.804	0.376	0.370		
10	0.415	0.408	0.222	0.216	0.478	0.416	0.802	0.804	0.381	0.365		
mean, N	0.4082	20	0.2167	18	0.4228	20	0.7907	20	0.3734	18		
origin of target sd (σ_h)	perception											
abs. target sd (σ_h) & as RSD%	0.0155	3.8	0.0100	4.6	0.0833	19.7	0.1154	14.6	0.0377	10.1		
s_{an}	0.0076		0.0049		0.0416		0.0576		0.0187			
s_{an} / σ_h	0.4893		0.4904		0.4992		0.4988		0.4958			
$s_{an} / \sigma_h < 0.5?$	yes											
s_{sam}^2	0.00E+00		0.00E+00		1.00E-04		2.21E-04		0.00E+00			
σ_{all}^2	2.17E-05		8.94E-06		6.24E-04		1.20E-03		1.28E-04			
critical	9.89E-05		4.39E-05		2.92E-03		5.60E-03		6.36E-04			
$s_{sam}^2 < \text{critical?}$	ACCEPT											

Table 3.1: Homogeneity D/L Values for Mollusc Shell (B) 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	0.173	0.150	0.262	0.253	0.236	0.233	0.306	0.221
2	0.168	0.169	0.258	0.257	0.219	0.243	0.299	0.288
3	0.171	0.160	0.260	0.249	0.252	0.229	0.296	0.270
4	0.160	0.159	0.254	0.254	0.245	0.253	0.284	0.284
5	0.183	0.149	0.254	0.255	0.203	0.253	0.239	0.232
6	0.166	0.167	0.256	0.257	0.225	0.231	0.295	0.292
7	0.157	0.162	0.244	0.256	0.229	0.222	0.261	0.295
8	0.140	0.168	0.221	0.254	C 0.237	0.248	0.213	0.279
9	0.169	0.161	0.255	0.254	0.222	0.239	0.237	0.277
10	0.172	0.166	0.260	0.251	0.242	0.234	0.305	0.282
mean, N	0.1634	20	0.2549	18	0.2347	20	0.2727	20
origin of target sd (σ_h)	perception		perception		perception		perception	
abs. target sd (σ_h) & as RSD%	0.0235	14.4	0.0102	4.0	0.0293	12.5	0.0573	21.0
s_{an}	0.0117		0.0050		0.0147		0.0281	
s_{an} / σ_h	0.4966		0.4927		0.4996		0.4911	
$s_{an} / \sigma_h < 0.5?$	yes		yes		yes		yes	
s_{sam}^2	0.00E+00		0.00E+00		0.00E+00		4.84E-05	
σ_{all}^2	4.98E-05		9.35E-06		7.75E-05		2.95E-04	
critical	2.32E-04		4.61E-05		3.63E-04		1.35E-03	
$s_{sam}^2 < \text{critical?}$	ACCEPT		ACCEPT		ACCEPT		ACCEPT	

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

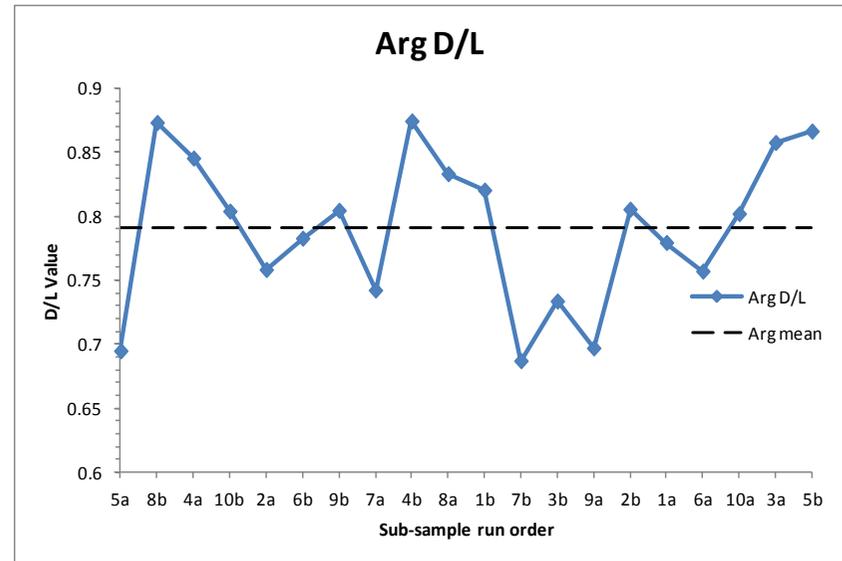
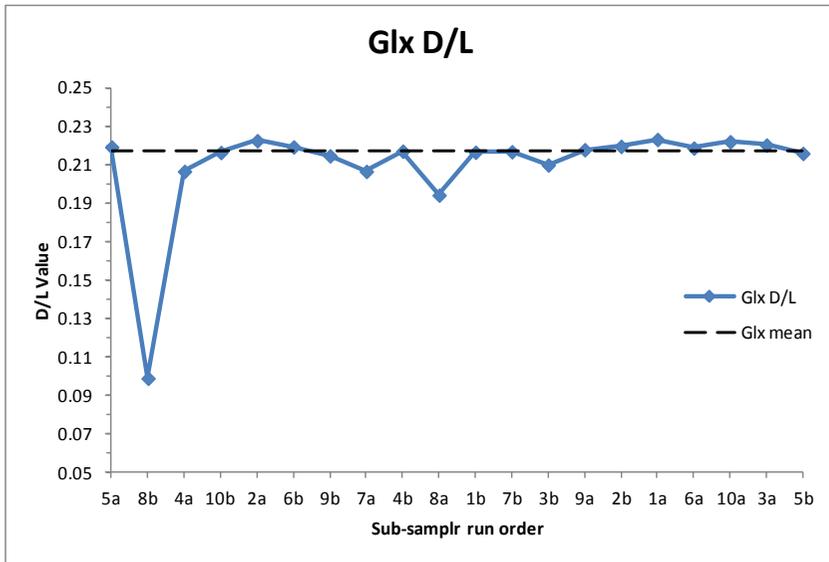
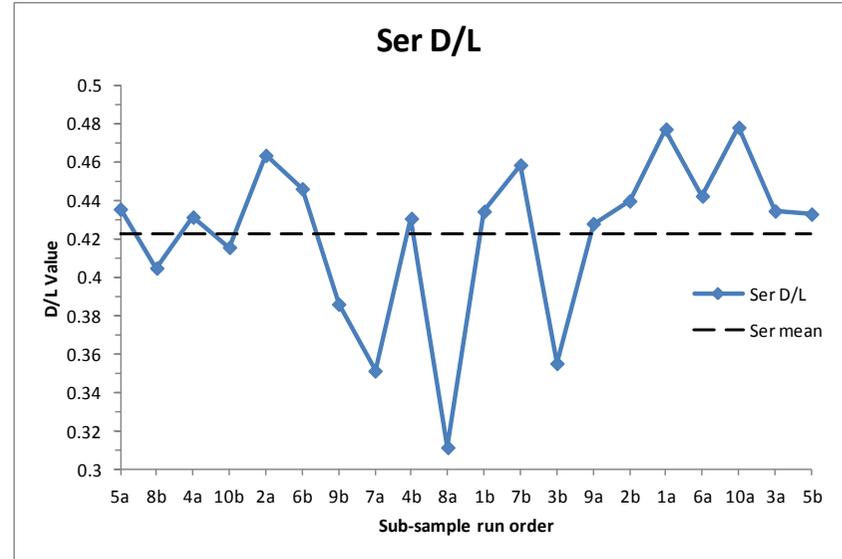
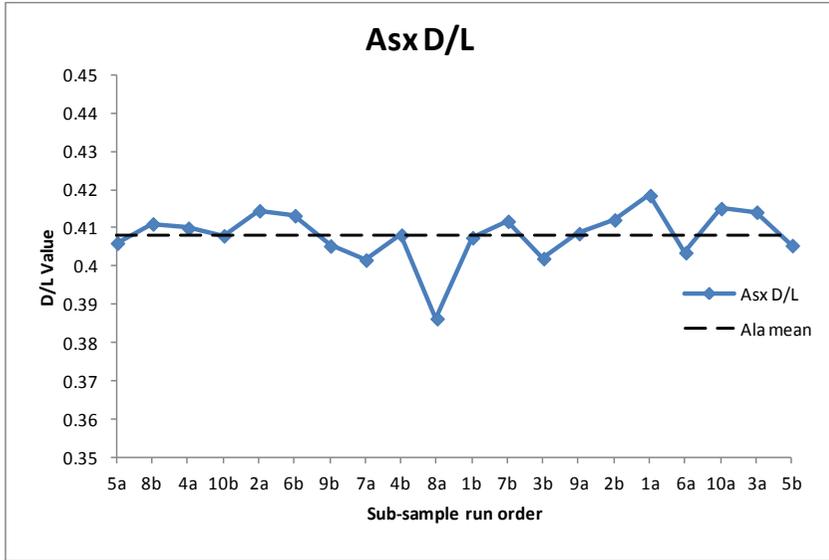


Figure 3.1: Homogeneity Amino Acid D/L Values in Analytical Sequence Order (continued).

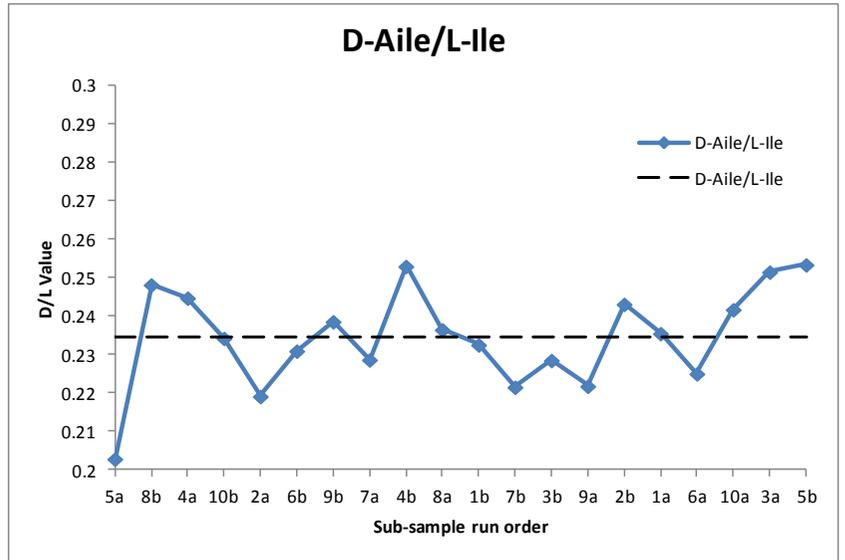
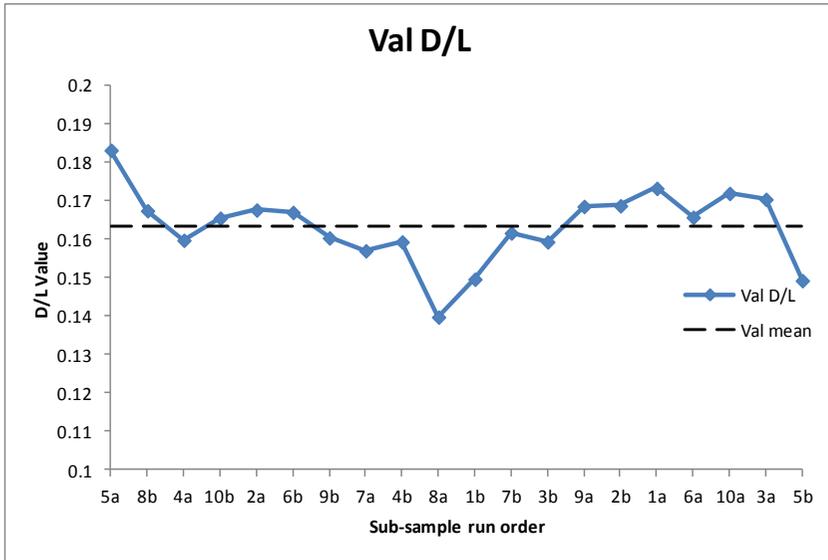
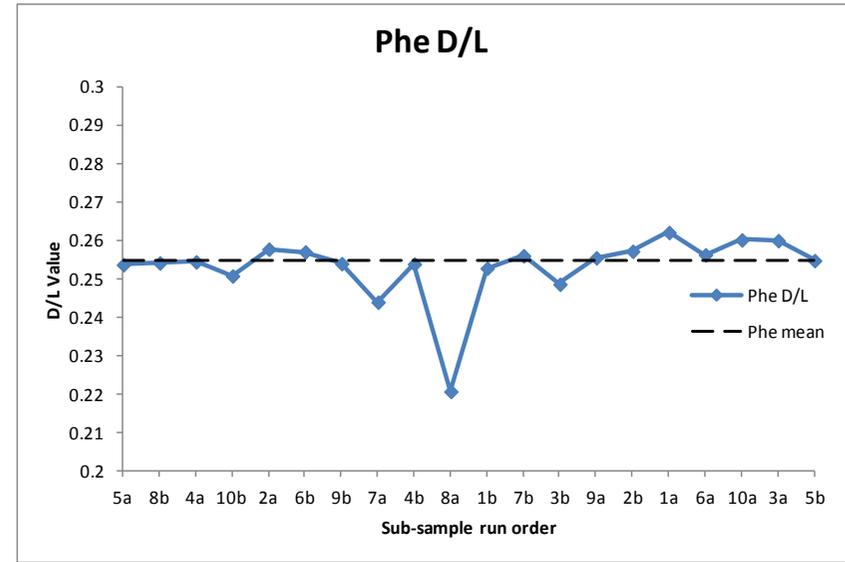
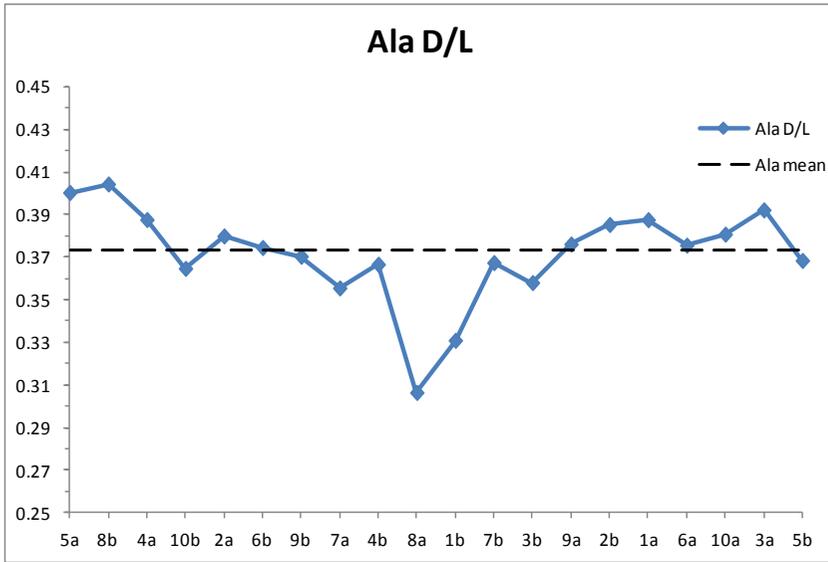


Figure 3.1: Homogeneity Amino Acid D/L Values in Analytical Sequence Order; (continued)

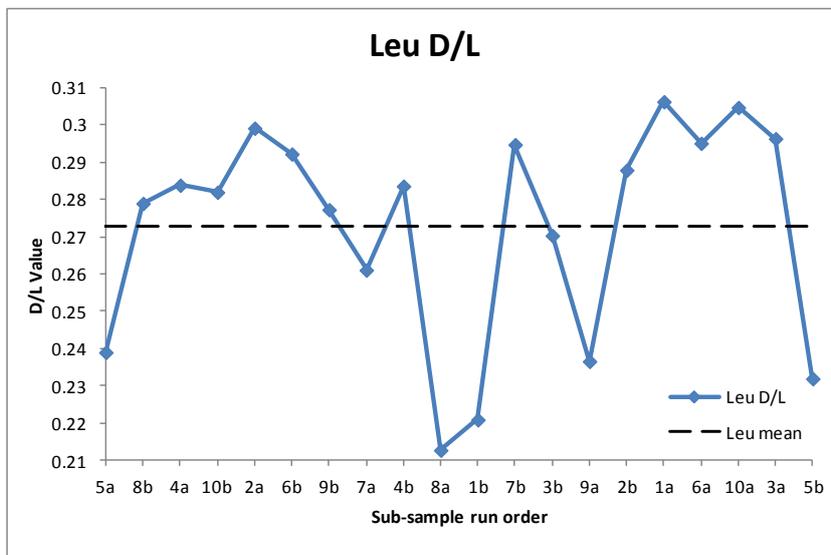


Figure 3.2: Homogeneity Amino Acid D/L Values; Paired Sub-samples showing Outliers.

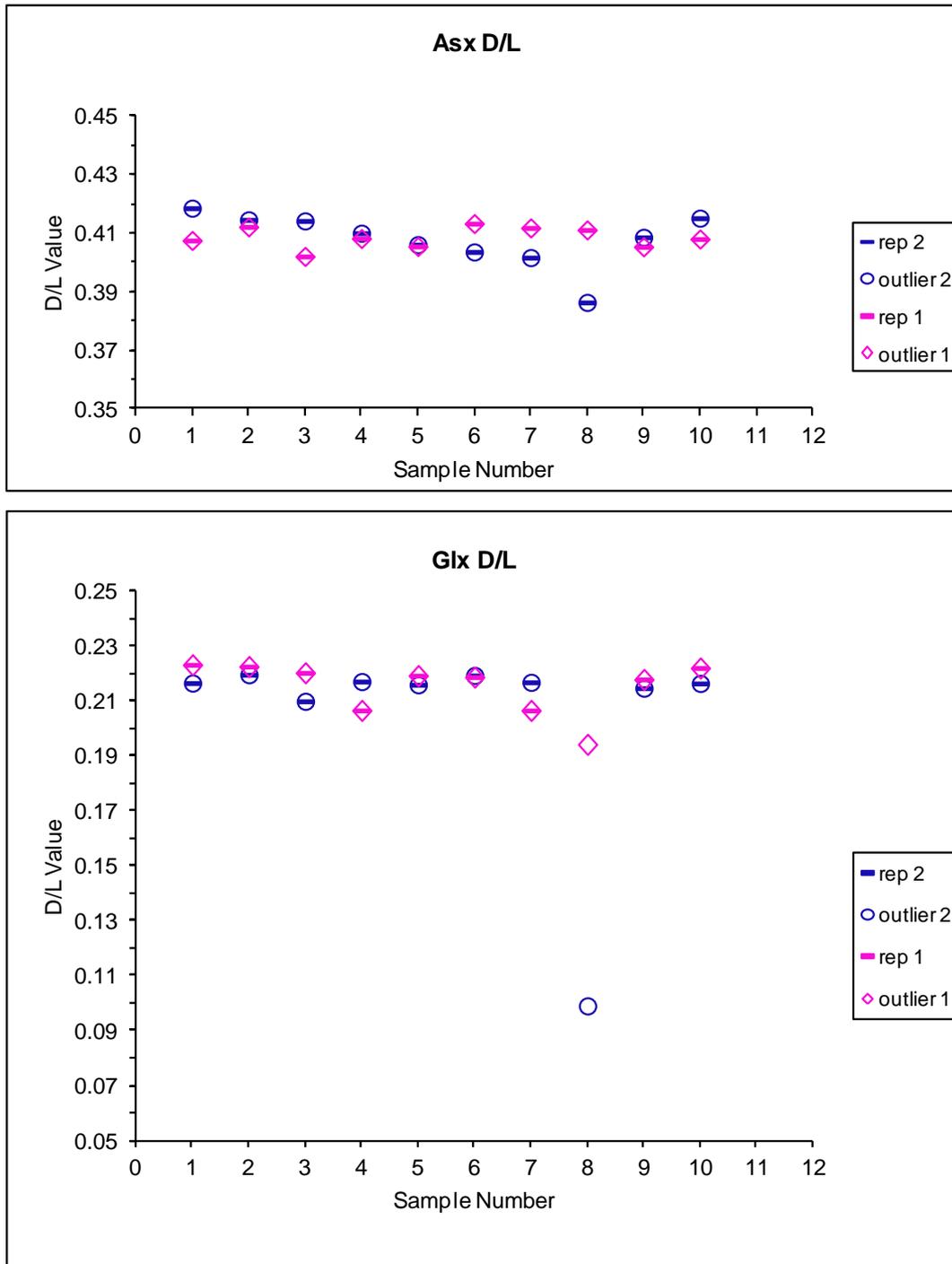


Figure 3.2: Homogeneity Amino Acid D/L Values; Paired Sub-samples showing Outliers.

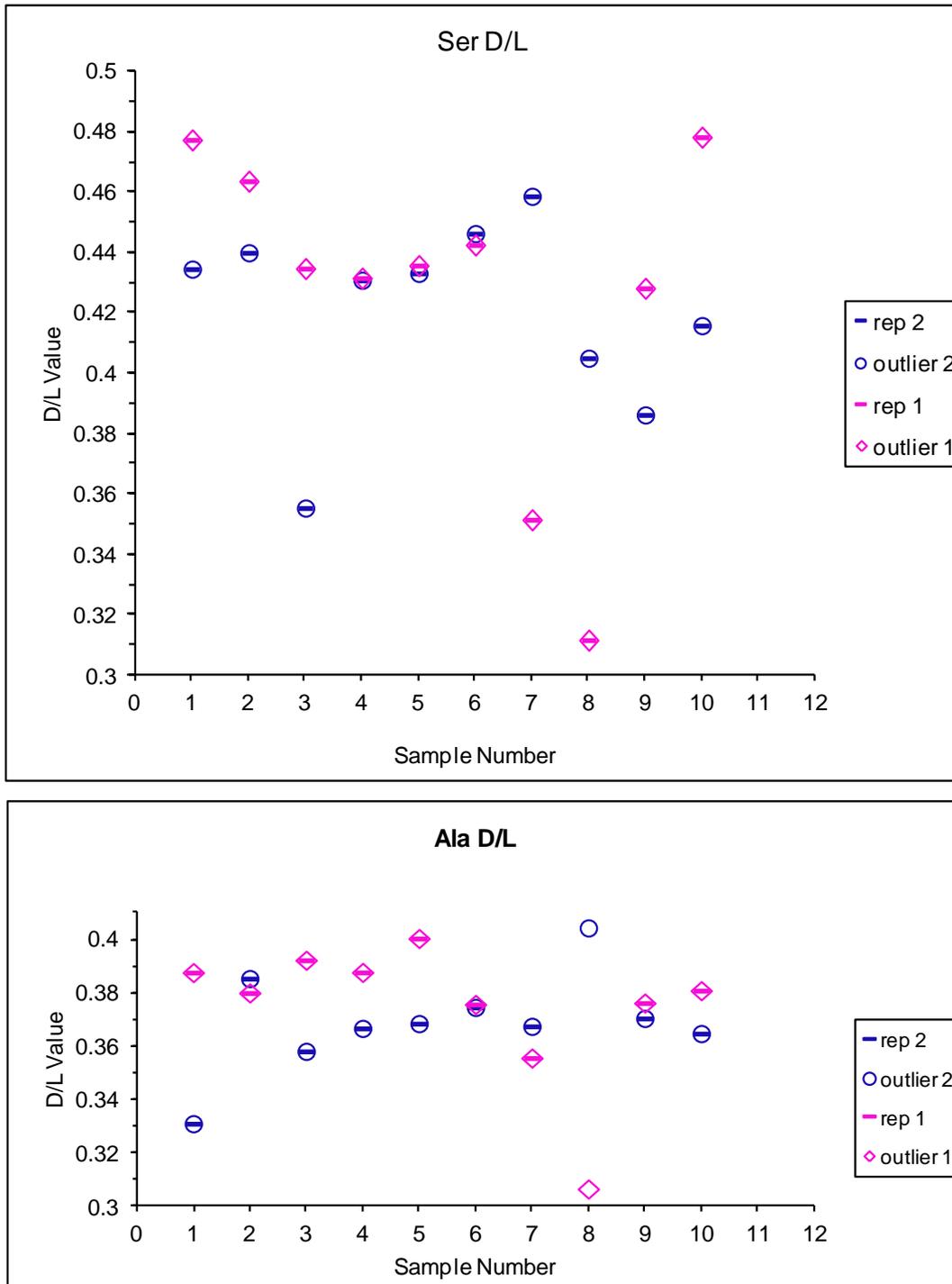


Figure 3.2: Homogeneity Amino Acid D/L Values; Paired Sub-samples showing Outliers.

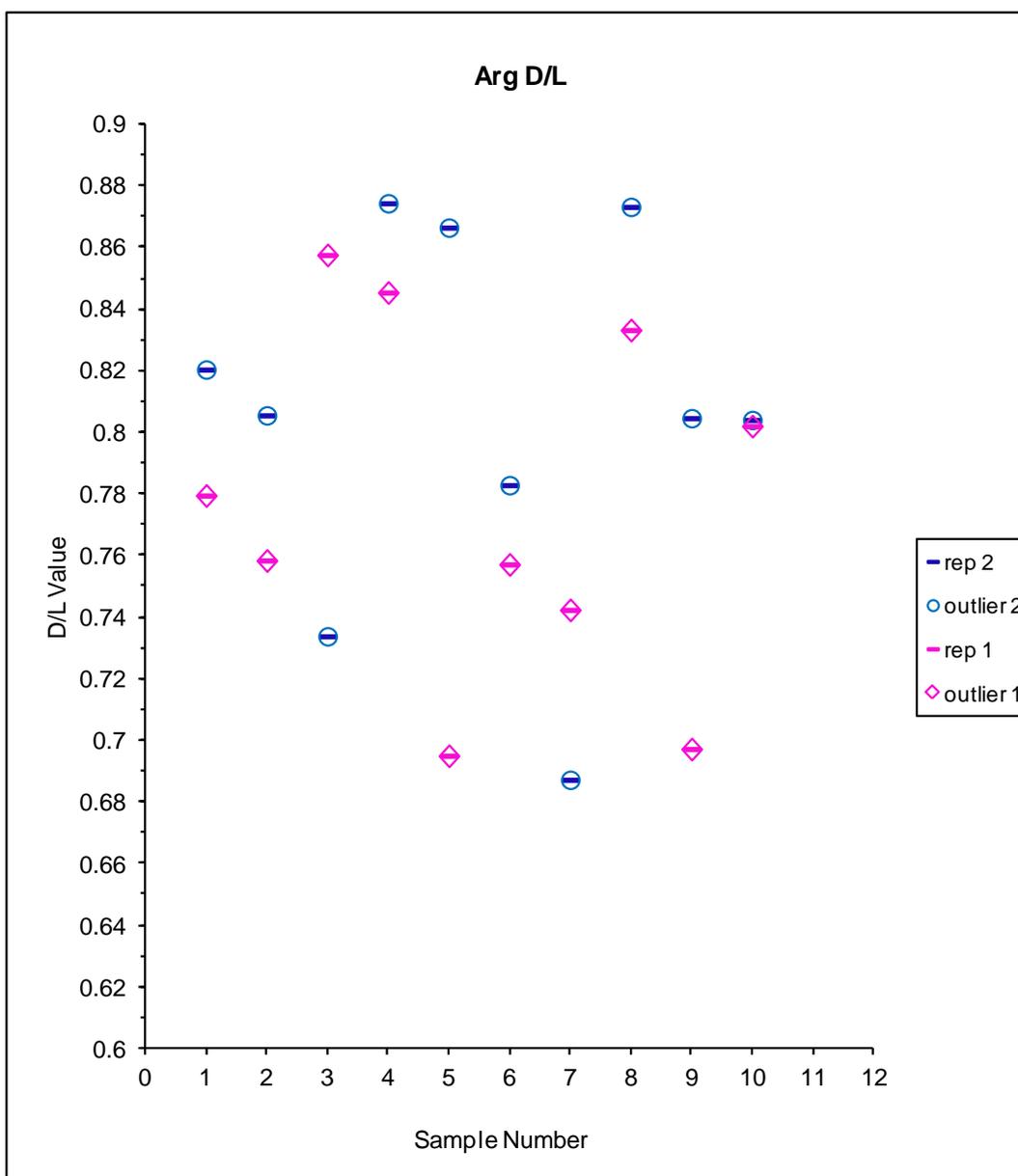


Figure 3.2: Homogeneity Amino Acid D/L Values; Paired Sub-samples showing Outliers.

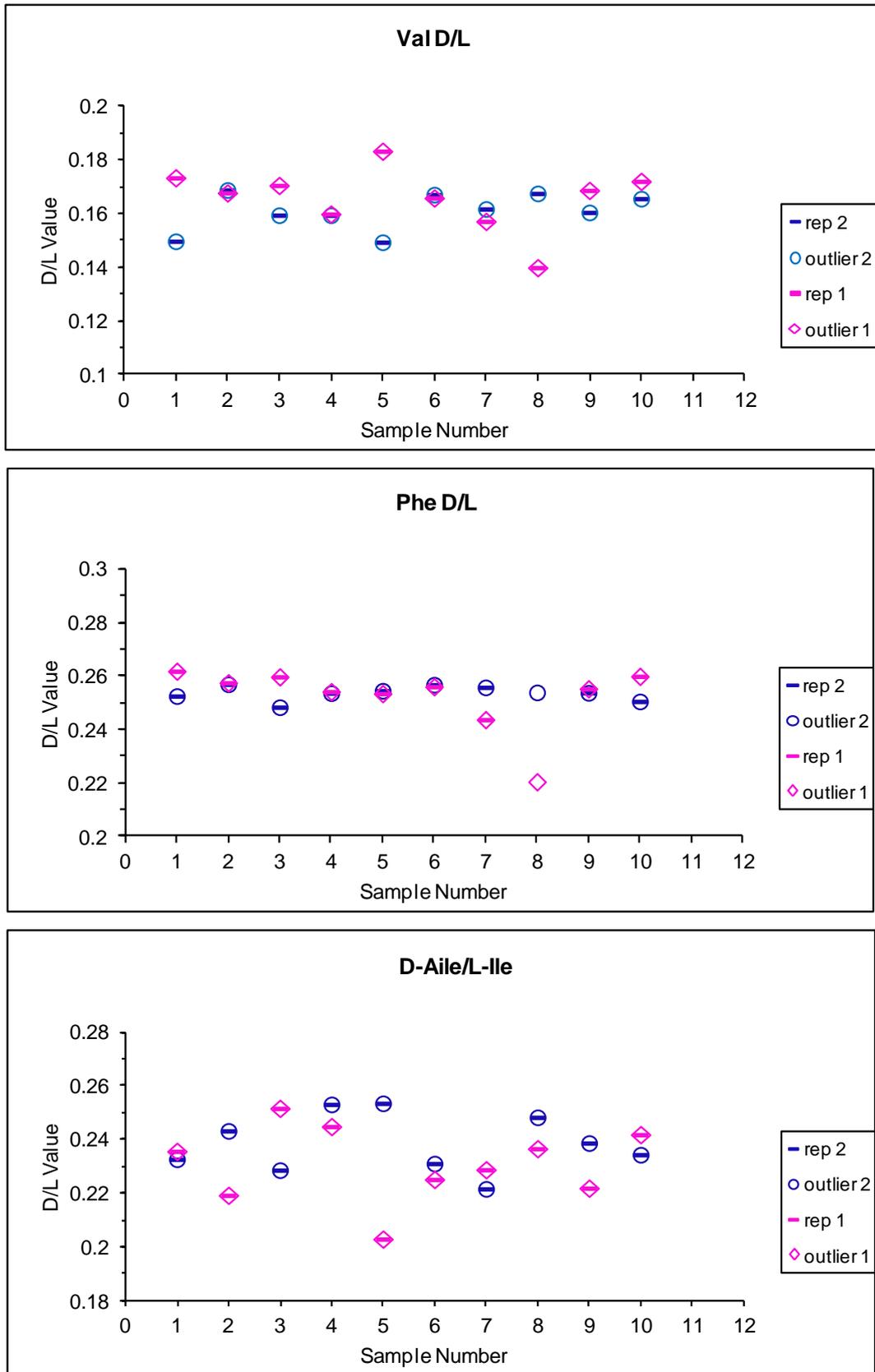
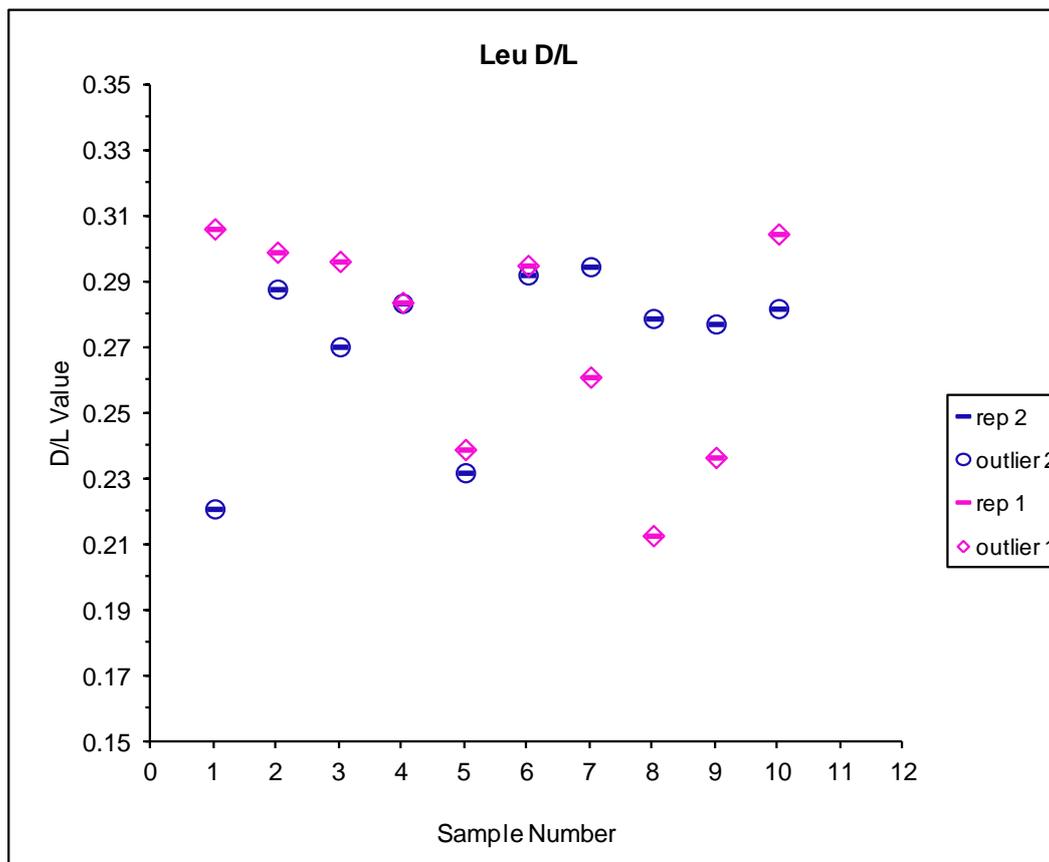


Figure 3.2: Homogeneity Amino Acid D/L Values; Paired Sub-samples showing Outliers.



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 trial 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;

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

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

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

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	12	29	0.392	0.0020	0.52	0.0186	4.74	0.0187	4.77
Asx D/L-rpHPLC	11	28	0.391	0.0020	0.52	0.0185	4.73	0.0186	4.76
Glx D/L-all ^a	12	29	0.189	0.0020	1.08	0.0278	14.67	0.0279	14.71
Glx D/L-rpHPLC	11	28	0.189	0.0020	1.08	0.0284	15.02	0.0285	15.06
Ser D/L-rpHPLC	11	28	0.393	0.0091	2.32	0.0676	17.22	0.0682	17.37
Arg D/L-rpHPLC	8	14	0.650	0.1566	24.10	0.0585	9.00	0.1672	25.73
Ala D/L-all ^a	13	30	0.360	0.0085	2.35	0.0210	5.82	0.0226	6.28
Ala D/L-rpHPLC	11	28	0.356	0.0085	2.38	0.0122	3.43	0.0149	4.18
Val D/L-all ^a	12	29	0.165	0.0094	5.68	0.0152	9.24	0.0179	10.85
Val D/L-rpHPLC	11	28	0.166	0.0094	5.64	0.0134	8.06	0.0164	9.84
Phe D/L-alla	12	29	0.235	0.0195	8.31	0.0292	12.42	0.0351	14.94
Phe D/L-rpHPLC	11	28	0.235	0.0195	8.30	0.0301	12.80	0.0359	15.26
D-Aile/L-Ile -allb	14	33	0.187	0.0194	10.38	0.0614	32.92	0.0644	34.52
D-Aile/L-Ile -rpHPLC	11	28	0.192	0.0204	10.61	0.0667	34.77	0.0698	36.35
D-Aile/L-Ile -HPLC-IE	2	4	0.154	0.0060	3.91	<S _r			
D-Aile/L-Ile -GC				Not determined					
Leu D/L-alla	9	24	0.241	0.0318	13.21	0.0325	13.49	0.0455	18.88
Leu D/L-rpHPLC	7	22	0.246	0.0318	12.94	0.0303	12.30	0.0439	17.86
Tyr D/L-rpHPLC	5	9	0.216	0.0041	1.91	0.0094	4.37	0.0103	4.77

^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;

$$RSD\% \text{ or } CV\% = \left(\frac{s}{\bar{x}} \right) \times 100$$

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. **Note: 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; $RSU\% = \left(\frac{u(\bar{x})}{\bar{x}} \right) \times 100$

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;

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

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, k , 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;

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

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 ($\alpha=0.05$), $t=3.18$ 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;

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

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 of 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 be much smaller than the contribution from method precision between different samples. It therefore makes more sense to put time into increasing the number of 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).

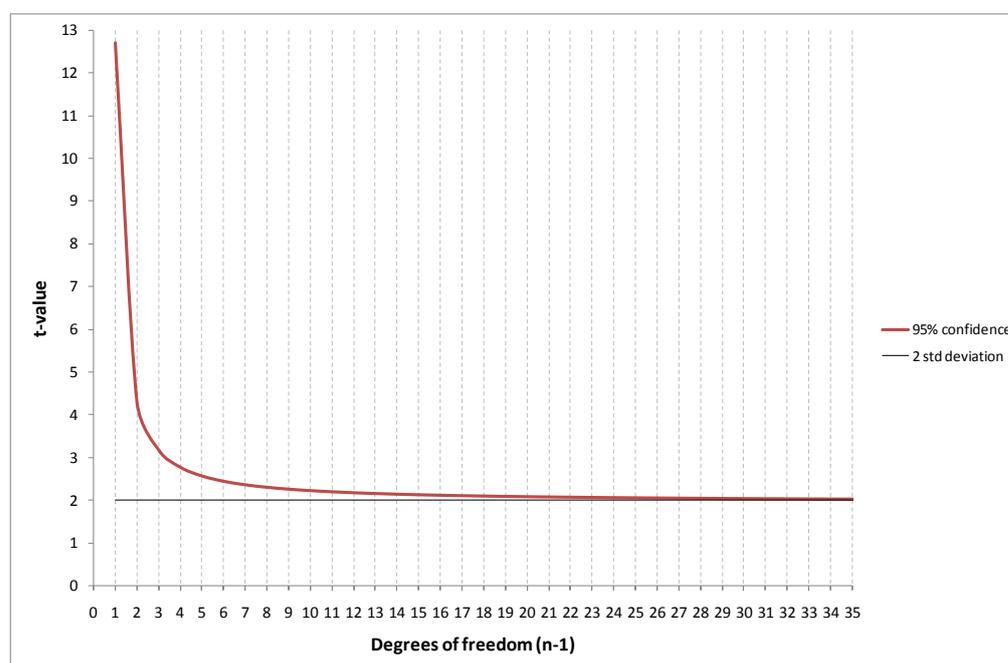


Table 4.2: Summary Statistics for L and D Aspartic Acid / Asparagine Peak Area Data

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)
1	RP	18679	18795	18913	19345	19879	19906	20523	20073	20348	20376	7947	10	697.0	8.77	220.4	2.77	5.55	2.262	6.27
2	RP	2403	10810	10631								7948	3	4803.0	60.43	2773.0	34.89	69.78	4.303	150.12
3	RP	12353										12353	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	22442	22219									22330	2	158.1	0.71	111.8	0.50	1.00	12.710	6.36
9	RP	9749	9729									9739	2	13.9	0.14	9.9	0.10	0.20	12.710	1.29
10	RP	5436	5676									5556	2	169.3	3.05	119.7	2.16	4.31	12.710	27.39
11	RP	9742	10069									9906	2	231.1	2.33	163.4	1.65	3.30	12.710	20.97
12	RP	16199	15599									15899	2	424.8	2.67	300.4	1.89	3.78	12.710	24.02
13	RP	25318										25318	1							
14	RP	9287										9287	1							
15	RP	9599	9477									9538	2	86.3	0.90	61.0	0.64	1.28	12.710	8.13
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	6927	6993	7004	7201	7374	7406	7610	7483	7567	7577	7314	10	263.0	3.60	83.2	1.14	2.27	2.262	2.57
2	RP	907	4178	4060								3048	3	1855.5	60.86	1071.2	35.14	70.28	4.303	151.20
3	RP	4719										4719	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	9020	9022									9021	2	1.6	0.02	1.1	0.01	0.02	12.710	0.16
9	RP	4013	3993									4003	2	14.0	0.35	9.9	0.25	0.49	12.710	3.14
10	RP	2241	2320									2281	2	55.9	2.45	39.5	1.73	3.47	12.710	22.04
11	RP	3917	4048									3982	2	93.1	2.34	65.8	1.65	3.31	12.710	21.01
12	RP	6684	6419									6551	2	186.9	2.85	132.2	2.02	4.04	12.710	25.64
13	RP	10219										10219	1							
14	RP	3747										3747	1							
15	RP	3887	3875									3881	2	8.5	0.22	6.0	0.16	0.31	12.710	1.97

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)
1	RP	1128	1129	1114	1145	1140	1153	1147	1155	1149	1143	1140	10	12.9	1.13	4.1	0.36	0.72	2.262	0.81
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	1247	1225									1236	2	15.3	1.24	10.8	0.88	1.75	12.710	11.15
9	RP	1238	1280									1259	2	29.7	2.36	21.0	1.67	3.33	12.710	21.19
10	RP	1298	1274									1286	2	16.9	1.31	11.9	0.93	1.86	12.710	11.79
11	RP	1140	1151									1146	2	7.6	0.67	5.4	0.47	0.94	12.710	6.00
12	RP	1012	1057									1034	2	32.4	3.13	22.9	2.21	4.42	12.710	28.11
13	RP	920										920	1							
14	RP	1151										1151	1							
15	RP	1199	1155									1177	2	31.2	2.65	22.1	1.88	3.75	12.710	23.86
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	418	420	412	426	423	429	425	431	427	425	424	10	5.5	1.29	1.7	0.41	0.81	2.262	0.92
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	501	498									499	2	2.6	0.52	1.8	0.36	0.73	12.710	4.63
9	RP	510	525									518	2	11.1	2.15	7.9	1.52	3.04	12.710	19.34
10	RP	535	521									528	2	10.1	1.91	7.1	1.35	2.70	12.710	17.14
11	RP	458	463									461	2	3.1	0.67	2.2	0.48	0.95	12.710	6.04
12	RP	417	435									426	2	12.6	2.95	8.9	2.08	4.17	12.710	26.49
13	RP	371										371	1							
14	RP	464										464	1							
15	RP	485	472									479	2	9.4	1.97	6.7	1.39	2.79	12.710	17.70

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)
1	RP	0.371	0.372	0.370	0.372	0.371	0.372	0.371	0.373	0.372	0.372	0.372	10	0.0008	0.21	0.0003	0.07	0.14	2.262	0.15
2	RP	0.378	0.387	0.382								0.382	3	0.0045	1.18	0.0026	0.68	1.36	4.303	2.93
3	RP	0.382										0.382	1							
4	IE																			
5	IE																			
6.1 ¹	GC _A	0.413										0.413	8	0.0240	5.81	0.0085	2.05	4.11	2.365	4.86
6.2	GC																			
8	RP	0.402	0.406									0.404	2	0.0028	0.70	0.0020	0.50	0.99	12.710	6.29
9	RP	0.412	0.410									0.411	2	0.0008	0.21	0.0006	0.15	0.29	12.710	1.85
10	RP	0.412	0.409									0.411	2	0.0024	0.60	0.0017	0.42	0.84	12.710	5.35
11	RP	0.402	0.402									0.402	2	0.0000	0.00	0.0000	0.00	0.01	12.710	0.04
12	RP	0.413	0.412									0.412	2	0.0007	0.18	0.0005	0.13	0.26	12.710	1.63
13	RP	0.404										0.404	1							
14	RP	0.403										0.403	1							
15	RP	0.405	0.409									0.407	2	0.0028	0.69	0.0020	0.48	0.97	12.710	6.16

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

GC_A = derived using peak area

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

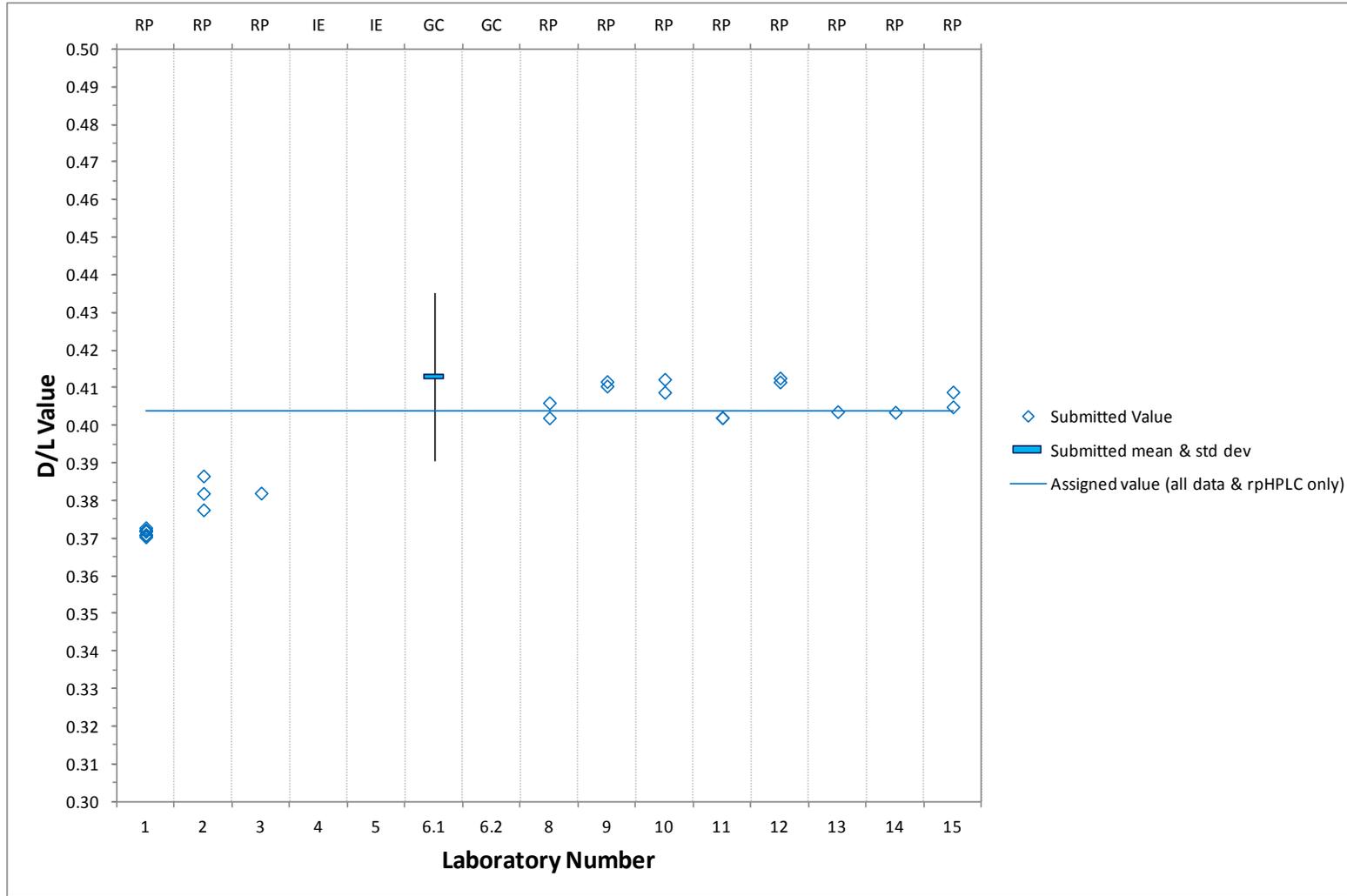


Figure 4.3: Experimental Expanded Uncertainty ($k=2$) of the Mean D/L value for Aspartic Acid / Asparagine (value of n displayed).

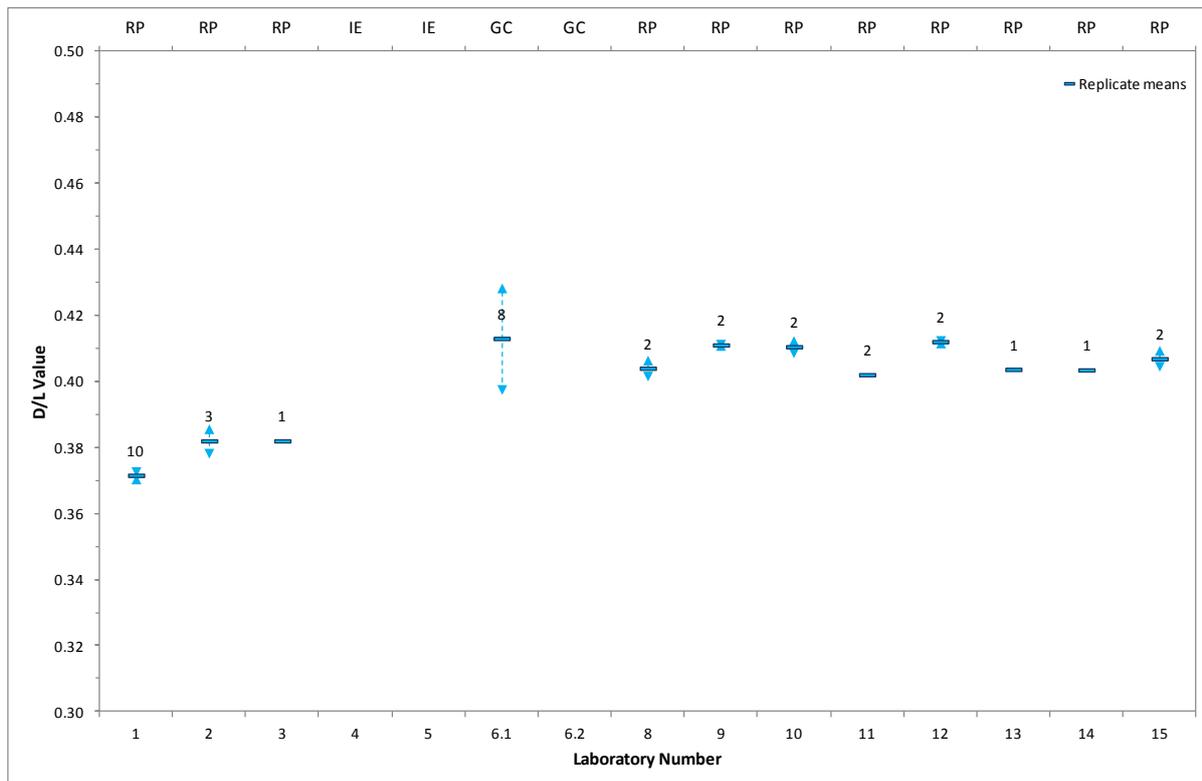


Figure 4.4: Experimental Expanded Uncertainty ($k=t_{(0.05,df)}$) of the Mean D/L value for Aspartic Acid / Asparagine (value of n displayed).

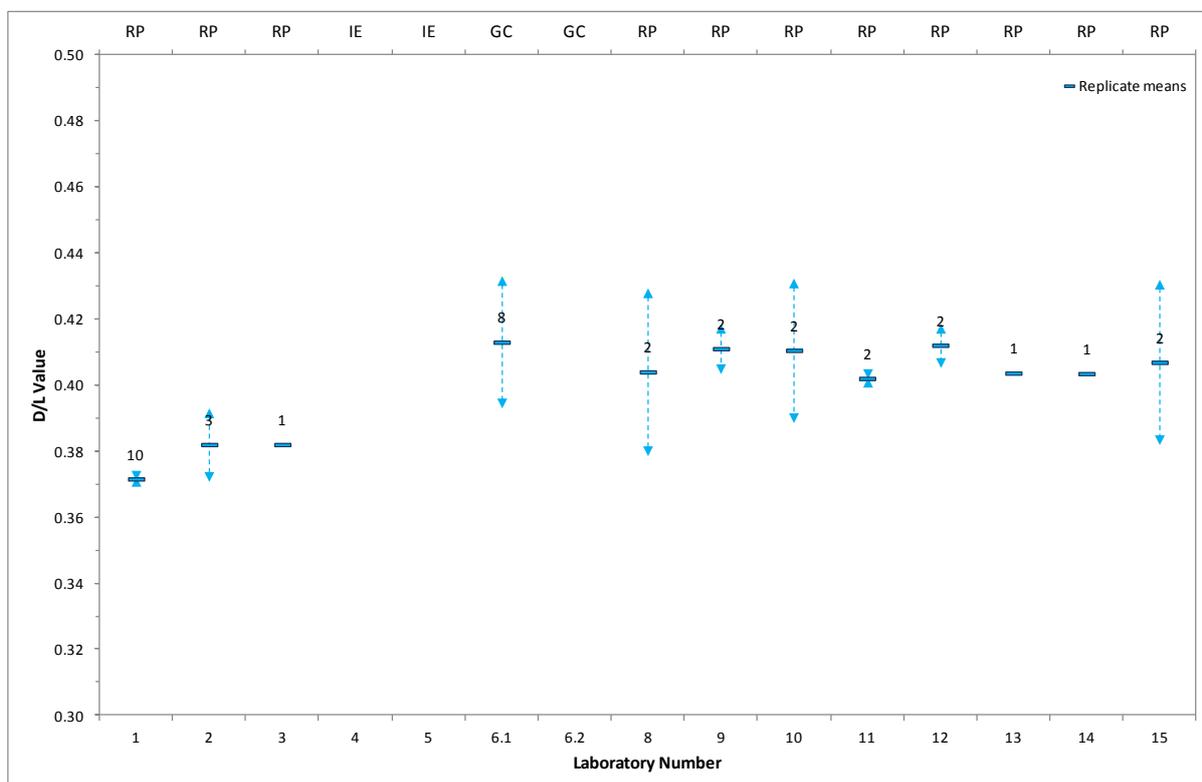


Table 4.5: Summary Statistics for L and D Glutamic Acid / Glutamine Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
		L-Glx peak area										mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% (k=t _{crit})
1	RP	8975	8943	9134	9253	9458	9415	9749	9318	9671	9650	9357	10	285.2	3.05	90.2	0.96	1.93	2.262	2.18
2	RP	864	3932	3873								2890	3	1754.8	60.73	1013.1	35.06	70.12	4.303	150.86
3	RP	4486										4486	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	8893	8403									8648	2	346.4	4.01	244.9	2.83	5.66	12.710	36.00
9	RP	3440	3395									3417	2	31.8	0.93	22.5	0.66	1.32	12.710	8.37
10	RP	1988	2005									1996	2	12.1	0.61	8.5	0.43	0.86	12.710	5.44
11	RP	3589	3702									3645	2	79.9	2.19	56.5	1.55	3.10	12.710	19.71
12	RP	5912	5704									5808	2	146.7	2.53	103.8	1.79	3.57	12.710	22.71
13	RP	9906										9906	1							
14	RP	3465										3465	1							
15	RP	3444	3445									3444	2	1.0	0.03	0.7	0.02	0.04	12.710	0.25
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	1392	1399	1434	1449	1481	1478	1536	1487	1502	1500	1466	10	46.3	3.16	14.6	1.00	2.00	2.262	2.26
2	RP	161	738	731								543	3	331.1	60.98	191.2	35.21	70.41	4.303	151.48
3	RP	836										836	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	1804	1786									1795	2	13.0	0.73	9.2	0.51	1.03	12.710	6.53
9	RP	738	726									732	2	8.5	1.16	6.0	0.82	1.63	12.710	10.38
10	RP	430	434									432	2	2.4	0.54	1.7	0.38	0.77	12.710	4.89
11	RP	759	775									767	2	11.5	1.50	8.1	1.06	2.12	12.710	13.49
12	RP	1274	1227									1250	2	33.0	2.64	23.3	1.87	3.73	12.710	23.73
13	RP	2122										2122	1							
14	RP	705										705	1							
15	RP	749	749									749	2	0.1	0.01	0.0	0.01	0.01	12.710	0.07

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										mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% (k=t _{crit})
		a	b	c	d	e	f	g	h	i	j									
1	RP	542	537	538	548	543	545	545	536	546	541	542	10	4.0	0.73	1.3	0.23	0.46	2.262	0.53
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	494	463									479	2	21.7	4.54	15.4	3.21	6.42	12.710	40.78
9	RP	456	466									461	2	7.2	1.57	5.1	1.11	2.22	12.710	14.10
10	RP	495	470									483	2	18.1	3.75	12.8	2.65	5.31	12.710	33.74
11	RP	439	442									440	2	2.3	0.53	1.6	0.37	0.75	12.710	4.74
12	RP	385	404									395	2	12.9	3.27	9.1	2.31	4.63	12.710	29.42
13	RP	376										376	1							
14	RP	448										448	1							
15	RP	449	438									444	2	7.6	1.72	5.4	1.22	2.44	12.710	15.48
		D-Glx Conc										mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% (k=t _{crit})
1	RP	84	84	84	86	85	86	86	86	85	84	85	10	0.7	0.85	0.2	0.27	0.54	2.262	0.61
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	100	98									99	2	1.3	1.26	0.9	0.89	1.78	12.710	11.32
9	RP	98	100									99	2	1.3	1.35	0.9	0.95	1.90	12.710	12.09
10	RP	107	102									104	2	4.0	3.82	2.8	2.70	5.40	12.710	34.29
11	RP	93	92									93	2	0.2	0.16	0.1	0.12	0.23	12.710	1.48
12	RP	83	87									85	2	2.7	3.16	1.9	2.23	4.47	12.710	28.40
13	RP	80										80	1							
14	RP	91										91	1							
15	RP	98	95									97	2	1.7	1.74	1.2	1.23	2.46	12.710	15.65

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)
1	RP	0.155	0.156	0.157	0.157	0.157	0.157	0.158	0.160	0.155	0.155	0.157	10	0.0013	0.84	0.0004	0.27	0.53	2.262	0.60
2	RP	0.186	0.188	0.189								0.187	3	0.0013	0.69	0.0007	0.40	0.80	4.303	1.71
3	RP	0.186										0.186	1							
4	IE																			
5	IE																			
6.1 ¹	GC _A	0.196										0.196	8	0.0040	2.04	0.0014	0.72	1.44	2.365	1.71
6.2	GC																			
8	RP	0.203	0.213									0.208	2	0.0071	3.40	0.0050	2.40	4.81	12.710	30.55
9	RP	0.214	0.214									0.214	2	0.0005	0.22	0.0003	0.16	0.32	12.710	2.01
10	RP	0.217	0.216									0.216	2	0.0001	0.06	0.0001	0.04	0.09	12.710	0.55
11	RP	0.211	0.209									0.210	2	0.0015	0.69	0.0010	0.49	0.98	12.710	6.22
12	RP	0.215	0.215									0.215	2	0.0002	0.11	0.0002	0.08	0.16	12.710	1.02
13	RP	0.214										0.214	1							
14	RP	0.203										0.203	1							
15	RP	0.218	0.218									0.218	2	0.0000	0.02	0.0000	0.01	0.03	12.710	0.18

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

GC_A = derived using peak area

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

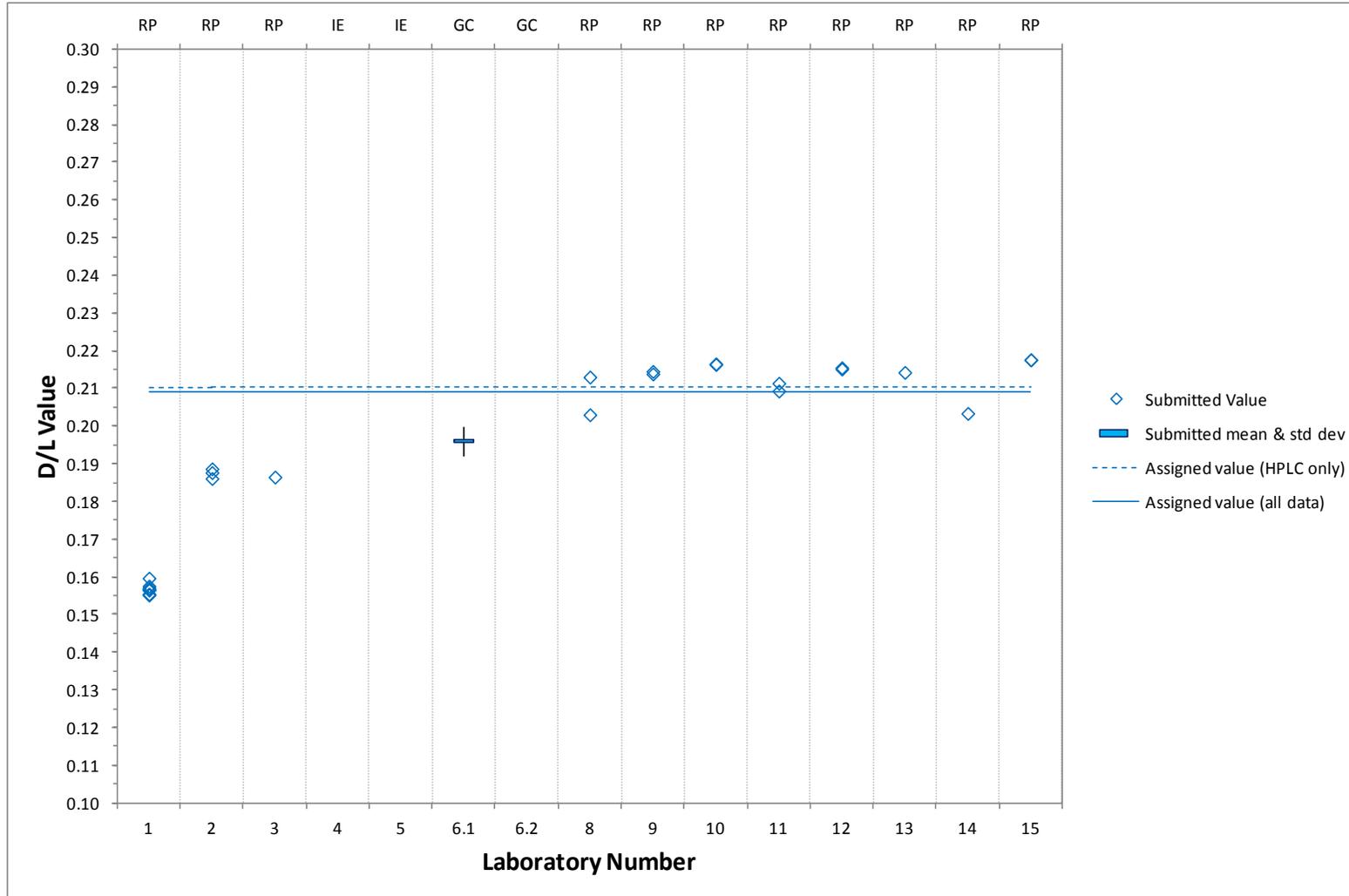


Figure 4.6: Experimental Expanded Uncertainty ($k=2$) of the Mean D/L value for **Glutamic Acid / Glutamine** (value of n displayed).

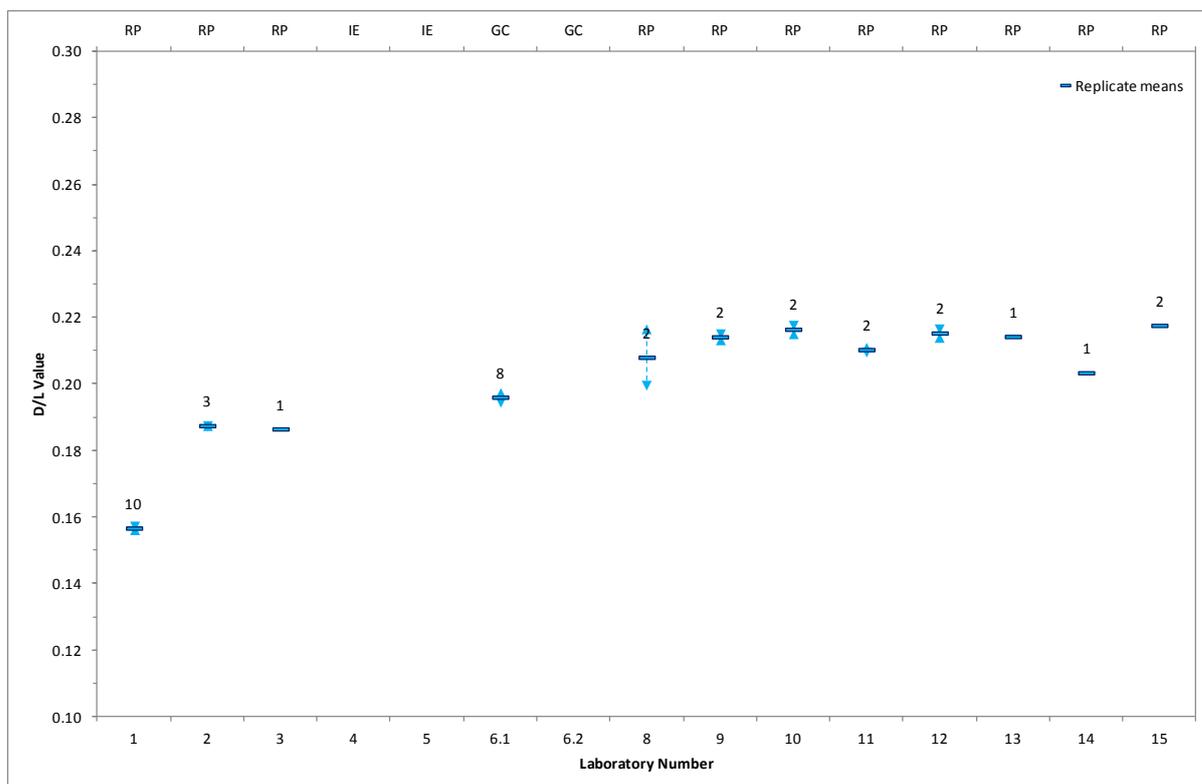


Figure 4.7: Experimental Expanded Uncertainty ($k=t_{(0.05,df)}$) of the Mean D/L value for **Glutamic Acid / Glutamine** (value of n displayed).

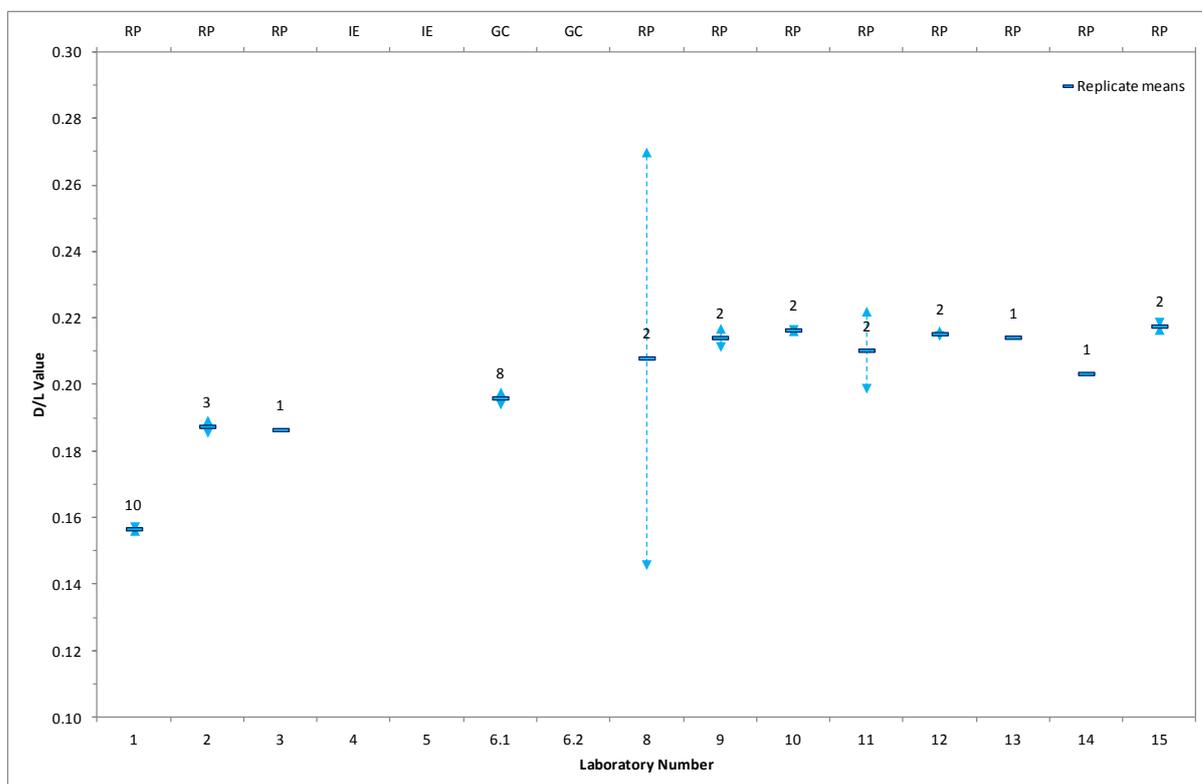


Table 4.8: Summary Statistics for L and D Serine Peak Area Data

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)
1	RP	2602	2587	2707	2699	2783	2743	2899	2774	2804	2836	2743	10	98.0	3.57	31.0	1.13	2.26	2.262	2.56
2	RP	218	994	1008								740	3	452.0	61.08	260.9	35.27	70.53	4.303	151.74
3	RP	1160										1160	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	2313	2041									2177	2	192.0	8.82	135.8	6.24	12.47	12.710	79.27
9	RP	872	857									864	2	10.4	1.20	7.3	0.85	1.70	12.710	10.79
10	RP	508	524									516	2	11.4	2.21	8.1	1.56	3.12	12.710	19.85
11	RP	1102	1115									1109	2	9.7	0.88	6.9	0.62	1.24	12.710	7.87
12	RP	1616	1554									1585	2	43.6	2.75	30.8	1.94	3.89	12.710	24.71
13	RP	2900										2900	1							
14	RP	1061										1061	1							
15	RP	887	838									862	2	34.5	4.00	24.4	2.83	5.66	12.710	35.94
D-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	832	824	873	868	895	871	905	883	894	909	875	10	28.7	3.28	9.1	1.04	2.07	2.262	2.35
2	RP	98	446	462								335	3	205.7	61.36	118.8	35.43	70.86	4.303	152.44
3	RP	478										478	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	1015	991									1003	2	17.5	1.74	12.4	1.23	2.47	12.710	15.68
9	RP	400	386									393	2	9.6	2.44	6.8	1.73	3.45	12.710	21.93
10	RP	230	230									230	2	0.3	0.12	0.2	0.08	0.17	12.710	1.07
11	RP	406	406									406	2	0.3	0.07	0.2	0.05	0.10	12.710	0.64
12	RP	699	666									683	2	23.7	3.46	16.7	2.45	4.90	12.710	31.14
13	RP	1265										1265	1							
14	RP	372										372	1							
15	RP	407	395									401	2	9.1	2.27	6.4	1.60	3.21	12.710	20.39

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)
1	RP	157	155	159	160	160	159	162	160	158	159	159	10	1.7	1.08	0.5	0.34	0.69	2.262	0.78
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	129	113									121	2	11.3	9.35	8.0	6.61	13.22	12.710	84.04
9	RP	115	117									116	2	1.5	1.30	1.1	0.92	1.84	12.710	11.69
10	RP	126	122									124	2	2.7	2.15	1.9	1.52	3.04	12.710	19.33
11	RP	134	132									133	2	1.1	0.79	0.7	0.56	1.12	12.710	7.10
12	RP	105	109									107	2	3.3	3.05	2.3	2.16	4.32	12.710	27.42
13	RP	109										109	1							
14	RP	136										136	1							
15	RP	115	106									110	2	6.3	5.75	4.5	4.06	8.13	12.710	51.65
	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	50	50	51	51	51	50	51	51	50	51	51	10	0.6	1.16	0.2	0.37	0.73	2.262	0.83
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	56	55									56	2	1.3	2.28	0.9	1.61	3.22	12.710	20.46
9	RP	53	53									53	2	0.0	0.06	0.0	0.04	0.09	12.710	0.55
10	RP	57	54									55	2	2.5	4.48	1.8	3.17	6.33	12.710	40.24
11	RP	49	48									49	2	0.8	1.59	0.5	1.13	2.25	12.710	14.33
12	RP	45	47									46	2	1.1	2.34	0.8	1.65	3.30	12.710	20.99
13	RP	48										48	1							
14	RP	48										48	1							
15	RP	53	50									51	2	2.1	4.02	1.5	2.84	5.68	12.710	36.11

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)
1	RP	0.320	0.319	0.322	0.322	0.321	0.318	0.312	0.318	0.319	0.321	0.319	10	0.0029	0.90	0.0009	0.28	0.57	2.262	0.64
2	RP	0.449	0.449	0.458								0.452	3	0.0055	1.21	0.0032	0.70	1.40	4.303	3.01
3	RP	0.412										0.412	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	0.439	0.485									0.462	2	0.0325	7.04	0.0230	4.98	9.96	12.710	63.27
9	RP	0.458	0.450									0.454	2	0.0056	1.24	0.0040	0.88	1.75	12.710	11.14
10	RP	0.454	0.439									0.447	2	0.0104	2.33	0.0074	1.65	3.29	12.710	20.92
11	RP	0.368	0.364									0.366	2	0.0029	0.80	0.0021	0.57	1.14	12.710	7.23
12	RP	0.433	0.428									0.431	2	0.0031	0.72	0.0022	0.51	1.01	12.710	6.44
13	RP	0.436										0.436	1							
14	RP	0.351										0.351	1							
15	RP	0.460	0.471									0.465	2	0.0081	1.73	0.0057	1.22	2.45	12.710	15.56

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

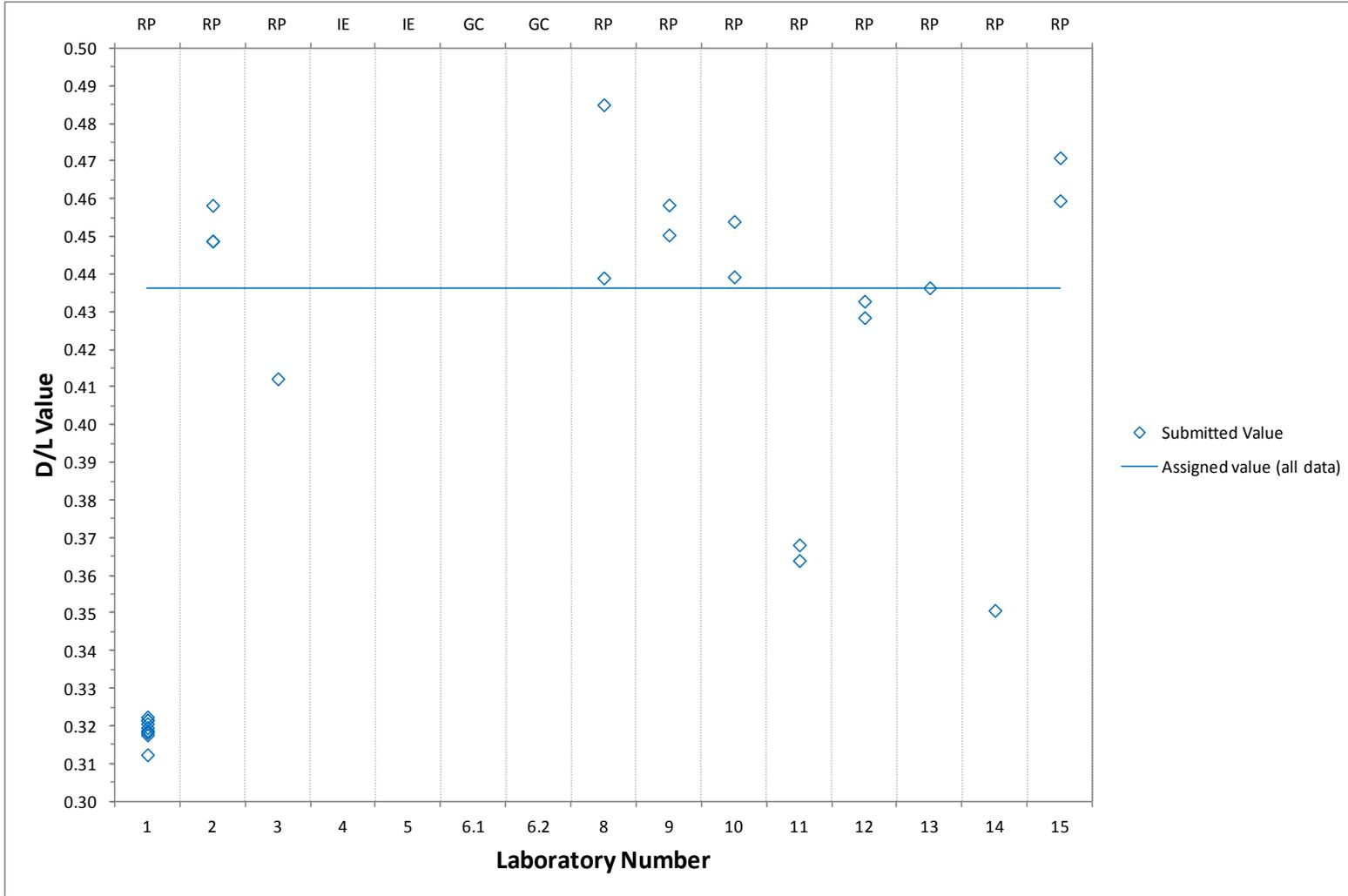


Figure 4.9: Experimental Expanded Uncertainty ($k=2$) of the Mean D/L value for Serine (value of n displayed).

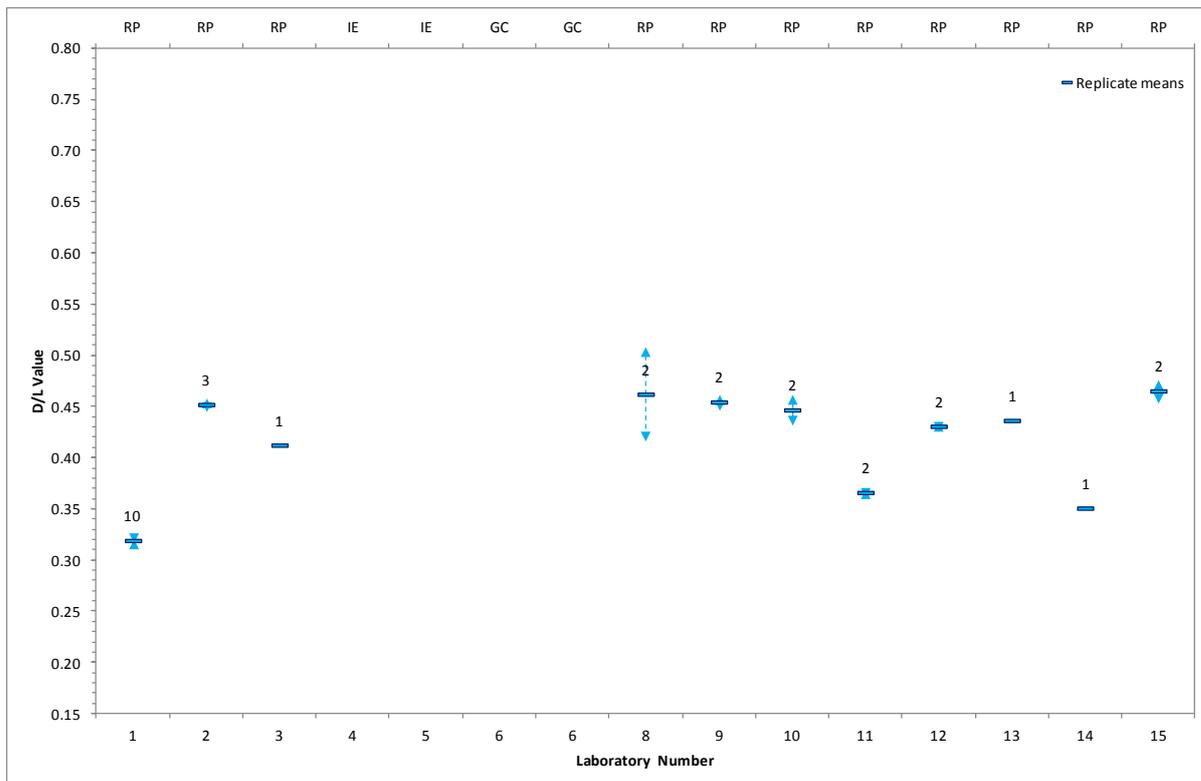


Figure 4.10: Experimental Expanded Uncertainty ($k=t_{(0.05,df)}$) of the Mean D/L value for Serine (value of n displayed).

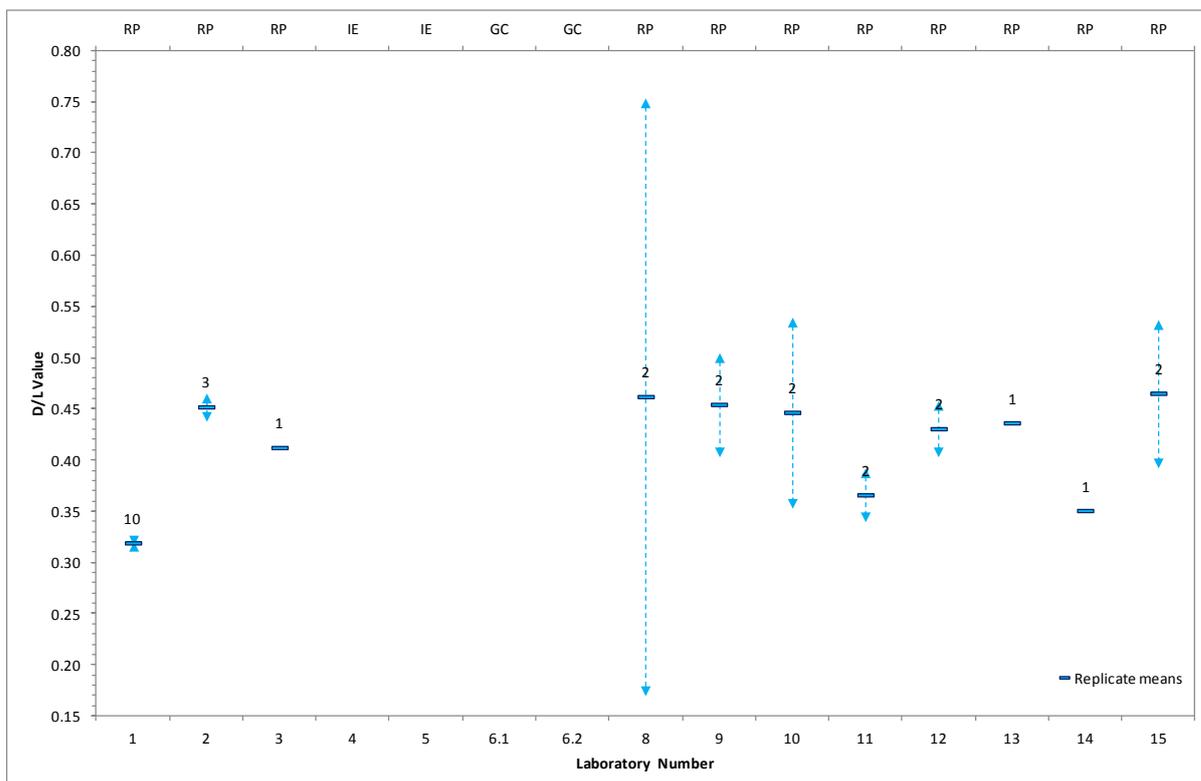


Table 4.11: Summary Statistics for L and D Arginine Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
		L-Arg peak area										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	264	1204	1186								885	3	537.4	60.73	310.2	35.06	70.12	4.303	150.86
3	RP	1410										1410	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP																			
9	RP	1218	1167									1192	2	36.3	3.04	25.7	2.15	4.31	12.710	27.36
10	RP	646	656									651	2	6.5	0.99	4.6	0.70	1.40	12.710	8.92
11	RP																			
12	RP	2052	1968									2010	2	59.8	2.98	42.3	2.11	4.21	12.710	26.76
13	RP	3557										3557	1							
14	RP	1082										1082	1							
15	RP	1128	1135									1131	2	4.9	0.44	3.5	0.31	0.62	12.710	3.93
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	161	738	720								540	3	327.8	60.75	189.3	35.07	70.15	4.303	150.91
3	RP	997										997	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP																			
9	RP	725	1134									930	2	288.9	31.08	204.3	21.98	43.95	12.710	279.33
10	RP	304	445									375	2	99.2	26.49	70.2	18.73	37.46	12.710	238.06
11	RP																			
12	RP	1265	1765									1515	2	353.6	23.34	250.1	16.51	33.01	12.710	209.80
13	RP	1244										1244	1							
14	RP	901										901	1							
15	RP	747	552									650	2	137.6	21.18	97.3	14.97	29.95	12.710	190.31

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										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																			
8	RP																			
9	RP	158	157									157	2	0.9	0.54	0.6	0.38	0.77	12.710	4.89
10	RP	158	150									154	2	5.2	3.37	3.7	2.38	4.76	12.710	30.26
11	RP																			
12	RP	131	136									134	2	3.8	2.82	2.7	2.00	3.99	12.710	25.37
13	RP	132										132	1							
14	RP	137										137	1							
15	RP	144	141									143	2	1.9	1.31	1.3	0.93	1.86	12.710	11.80
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																			
8	RP																			
9	RP	94	152									123	2	41.3	33.45	29.2	23.65	47.31	12.710	300.64
10	RP	74	102									88	2	19.6	22.26	13.9	15.74	31.48	12.710	200.04
11	RP																			
12	RP	81	122									101	2	29.4	28.95	20.8	20.47	40.94	12.710	260.15
13	RP	46										46	1							
14	RP	114										114	1							
15	RP	95	69									82	2	18.8	22.88	13.3	16.18	32.36	12.710	205.66

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	0.610	0.613	0.607								0.610	3	0.0031	0.50	0.0018	0.29	0.58	4.303	1.25	
3	RP	0.707										0.707	1								
4	IE																				
5	IE																				
6.1	GC																				
6.2	GC																				
8	RP																				
9	RP	0.596	0.972									0.784	2	0.2662	33.96	0.1882	24.02	48.03	12.710	305.25	
10	RP	0.471	0.678									0.575	2	0.1467	25.53	0.1037	18.05	36.10	12.710	229.45	
11	RP																				
12	RP	0.616	0.897									0.757	2	0.1985	26.23	0.1403	18.55	37.10	12.710	235.74	
13	RP	0.350										0.350	1								
14	RP	0.833										0.833	1								
15	RP	0.663	0.487									0.575	2	0.1242	21.60	0.0878	15.28	30.55	12.710	194.15	

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

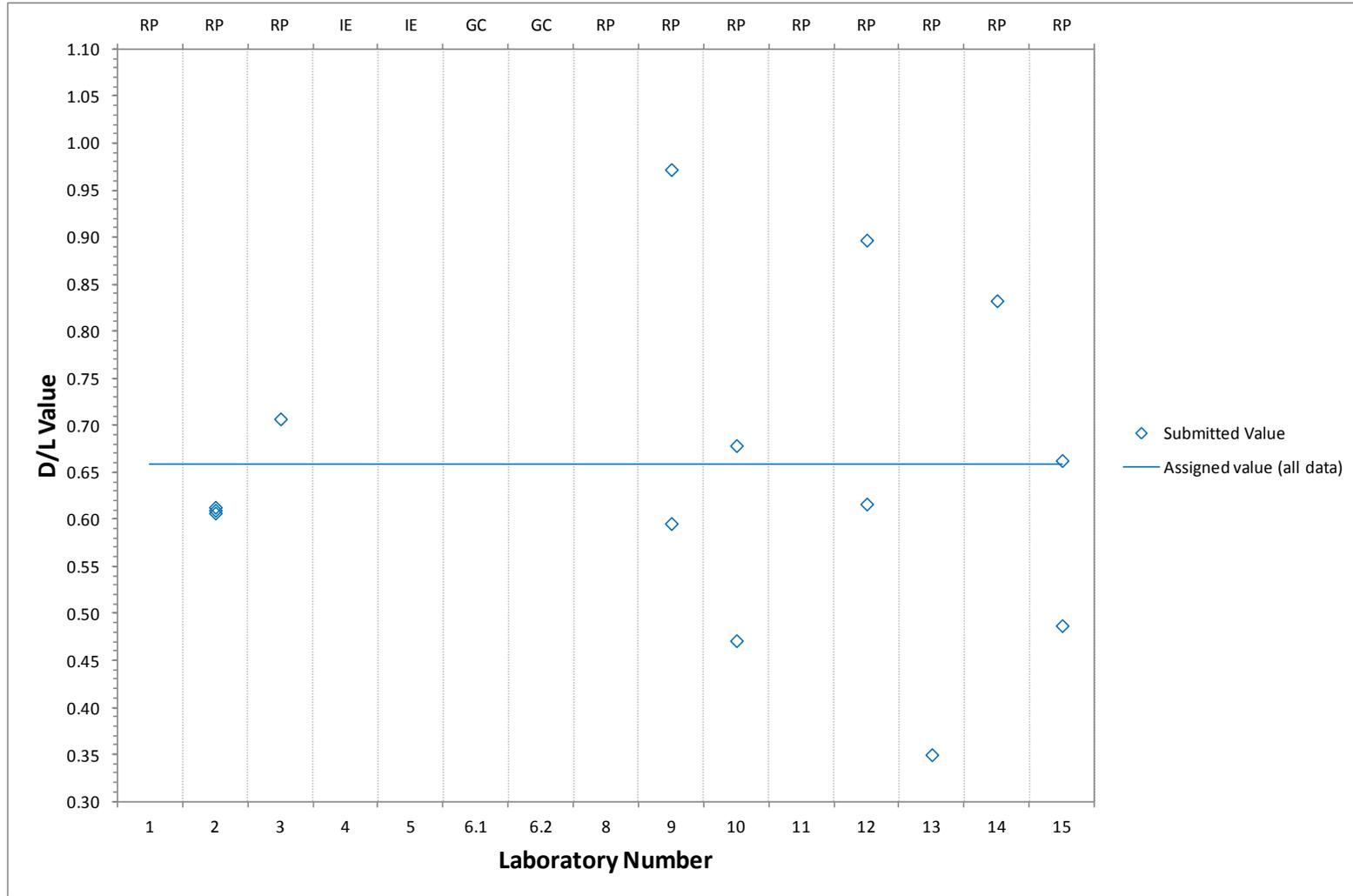


Figure 4.12: Experimental Expanded Uncertainty ($k=2$) of the Mean D/L value for Arginine (value of n displayed).

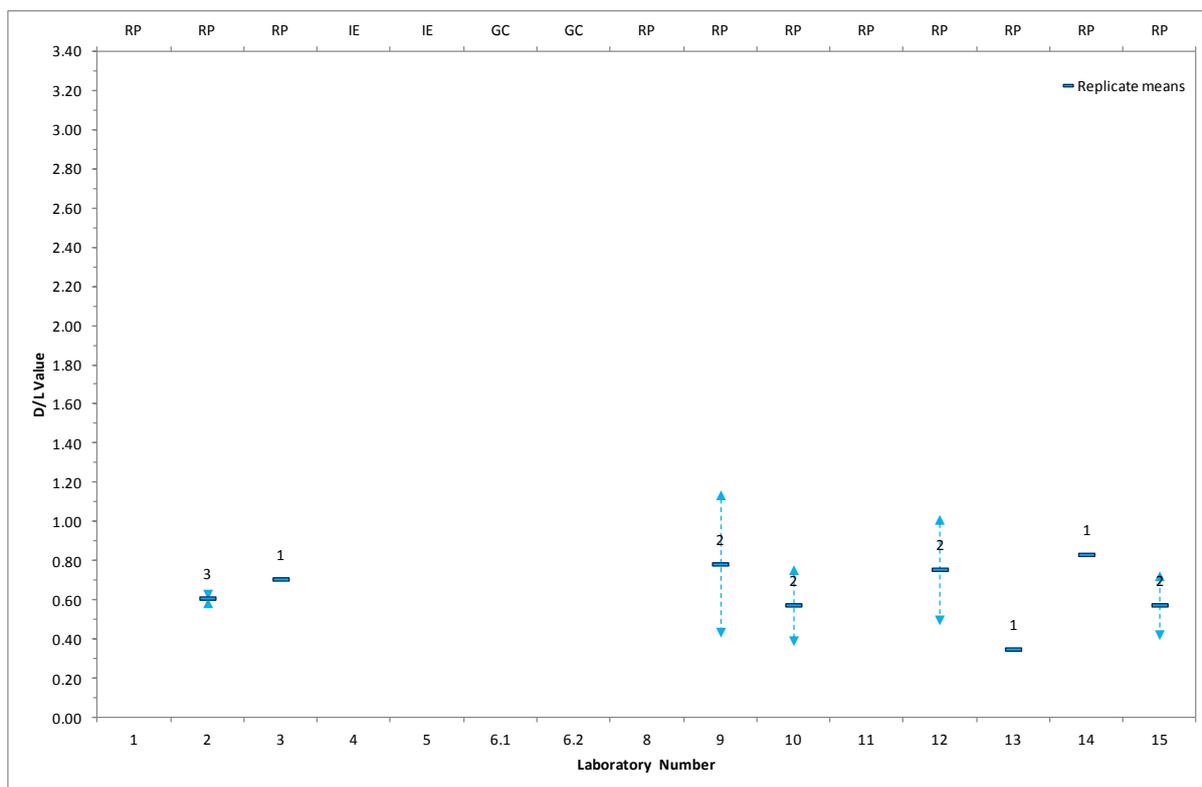


Figure 4.13: Experimental Expanded Uncertainty ($k=t_{(0.05,df)}$) of the Mean D/L value for Arginine (value of n displayed).

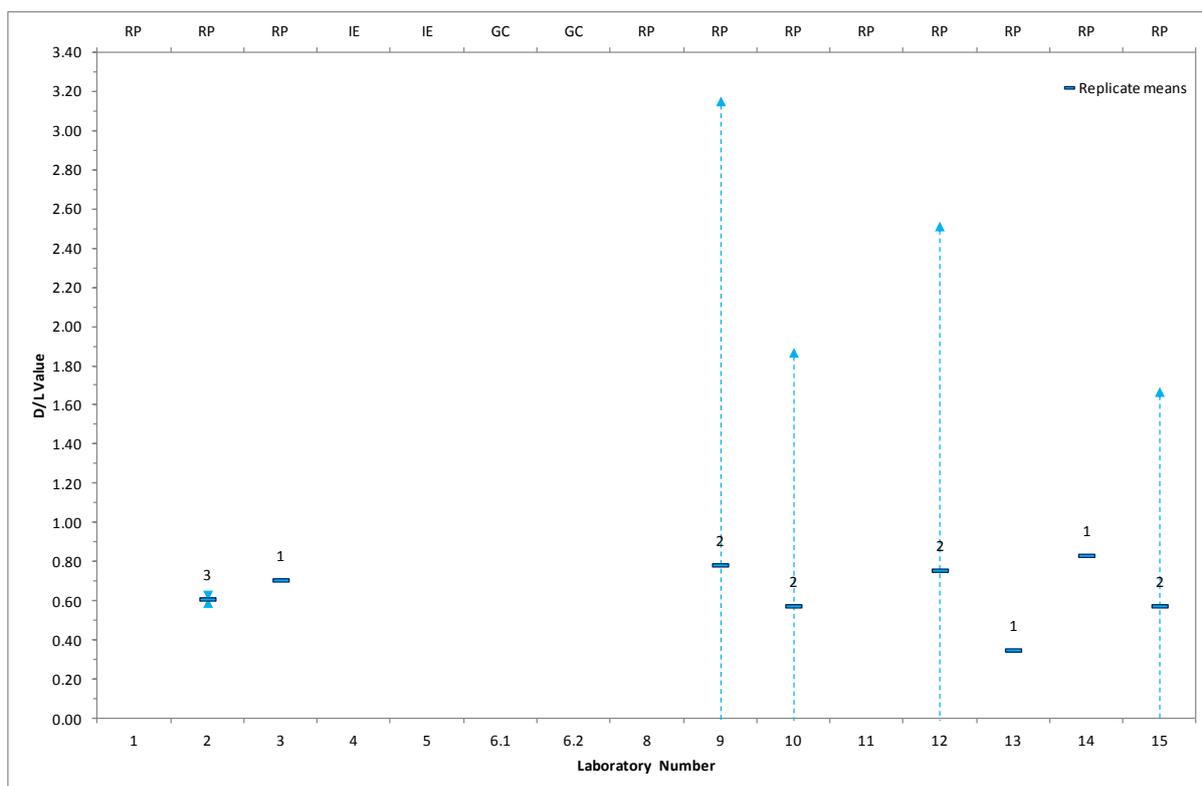


Table 4.14: Summary Statistics for L and D Alanine Peak Area Data

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)
1	RP	5399	5380	5589	5580	5684	5669	5836	5648	5702	5708	5620	10	140.2	2.49	44.3	0.79	1.58	2.262	1.78
2	RP	618	2851	2781								2083	3	1269.5	60.94	733.0	35.18	70.36	4.303	151.38
3	RP	3118										3118	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	6798	6387									6593	2	290.9	4.41	205.7	3.12	6.24	12.710	39.65
9	RP	2549	2456									2503	2	65.8	2.63	46.6	1.86	3.72	12.710	23.65
10	RP	1362	1402									1382	2	28.0	2.03	19.8	1.43	2.87	12.710	18.21
11	RP	2690	2741									2716	2	36.2	1.33	25.6	0.94	1.88	12.710	11.97
12	RP	4229	4043									4136	2	131.4	3.18	92.9	2.25	4.49	12.710	28.56
13	RP	6873										6873	1							
14	RP	2343										2343	1							
15	RP	2434	2480									2457	2	32.2	1.31	22.8	0.93	1.85	12.710	11.77
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	1972	2007	2039	1931	1992	1956	2061	1989	2064	2076	2009	10	49.6	2.47	15.7	0.78	1.56	2.262	1.77
2	RP	219	1023	1004								748	3	458.9	61.32	265.0	35.40	70.80	4.303	152.32
3	RP	1166										1166	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	2179	2175									2177	2	2.7	0.12	1.9	0.09	0.18	12.710	1.12
9	RP	1032	1006									1019	2	18.5	1.81	13.0	1.28	2.56	12.710	16.27
10	RP	533	562									548	2	20.5	3.74	14.5	2.65	5.29	12.710	33.62
11	RP	1141	1152									1146	2	7.8	0.68	5.5	0.48	0.97	12.710	6.14
12	RP	1916	1753									1834	2	115.7	6.30	81.8	4.46	8.92	12.710	56.66
13	RP	3102										3102	1							
14	RP	971										971	1							
15	RP	1022	1055									1038	2	23.5	2.26	16.6	1.60	3.20	12.710	20.33

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										mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% (k=t _{crit})
		a	b	c	d	e	f	g	h	i	j									
1	RP	326	323	329	330	326	328	326	325	322	320	326	10	3.2	0.98	1.0	0.31	0.62	2.262	0.70
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	378	352									365	2	18.0	4.94	12.8	3.50	6.99	12.710	44.43
9	RP	312	312									312	2	0.4	0.13	0.3	0.09	0.18	12.710	1.17
10	RP	314	304									309	2	7.2	2.33	5.1	1.65	3.30	12.710	20.97
11	RP	304	302									303	2	1.0	0.33	0.7	0.24	0.47	12.710	2.99
12	RP	255	264									260	2	6.8	2.62	4.8	1.85	3.71	12.710	23.57
13	RP	241										241	1							
14	RP	280										280	1							
15	RP	293	292									292	2	1.3	0.44	0.9	0.31	0.62	12.710	3.95
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	119	121	120	114	114	113	115	114	117	116	116	10	2.6	2.25	0.8	0.71	1.43	2.262	1.61
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	121	120									121	2	0.8	0.66	0.6	0.46	0.93	12.710	5.91
9	RP	107	108									108	2	0.7	0.69	0.5	0.49	0.98	12.710	6.20
10	RP	104	103									104	2	0.6	0.62	0.5	0.44	0.87	12.710	5.56
11	RP	109	108									108	2	1.1	0.98	0.8	0.69	1.39	12.710	8.83
12	RP	98	97									97	2	0.5	0.51	0.4	0.36	0.72	12.710	4.57
13	RP	92										92	1							
14	RP	98										98	1							
15	RP	104	105									105	2	0.5	0.51	0.4	0.36	0.72	12.710	4.61

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)
1	RP	0.365	0.373	0.365	0.346	0.350	0.345	0.353	0.352	0.362	0.364	0.358	10	0.0094	2.63	0.0030	0.83	1.66	2.262	1.88
2	RP	0.354	0.359	0.361								0.358	3	0.0036	1.02	0.0021	0.59	1.17	4.303	2.52
3	RP	0.374										0.374	1							
4	IE																			
5	IE																			
6.1 ¹	GC _A	0.405										0.405	4	0.0260	6.42	0.0130	3.21	6.42	3.182	10.22
6.2 ¹	GC _H	0.433										0.433	6	0.0320	7.39	0.0131	3.02	6.03	2.571	7.76
8	RP	0.321	0.341									0.331	2	0.0141	4.27	0.0100	3.02	6.04	12.710	38.40
9	RP	0.343	0.347									0.345	2	0.0028	0.82	0.0020	0.58	1.16	12.710	7.37
10	RP	0.332	0.340									0.336	2	0.0058	1.71	0.0041	1.21	2.43	12.710	15.41
11	RP	0.359	0.356									0.358	2	0.0023	0.65	0.0016	0.46	0.92	12.710	5.84
12	RP	0.384	0.367									0.376	2	0.0118	3.13	0.0083	2.21	4.43	12.710	28.13
13	RP	0.382										0.382	1							
14	RP	0.351										0.351	1							
15	RP	0.356	0.361									0.358	2	0.0034	0.95	0.0024	0.67	1.35	12.710	8.56

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

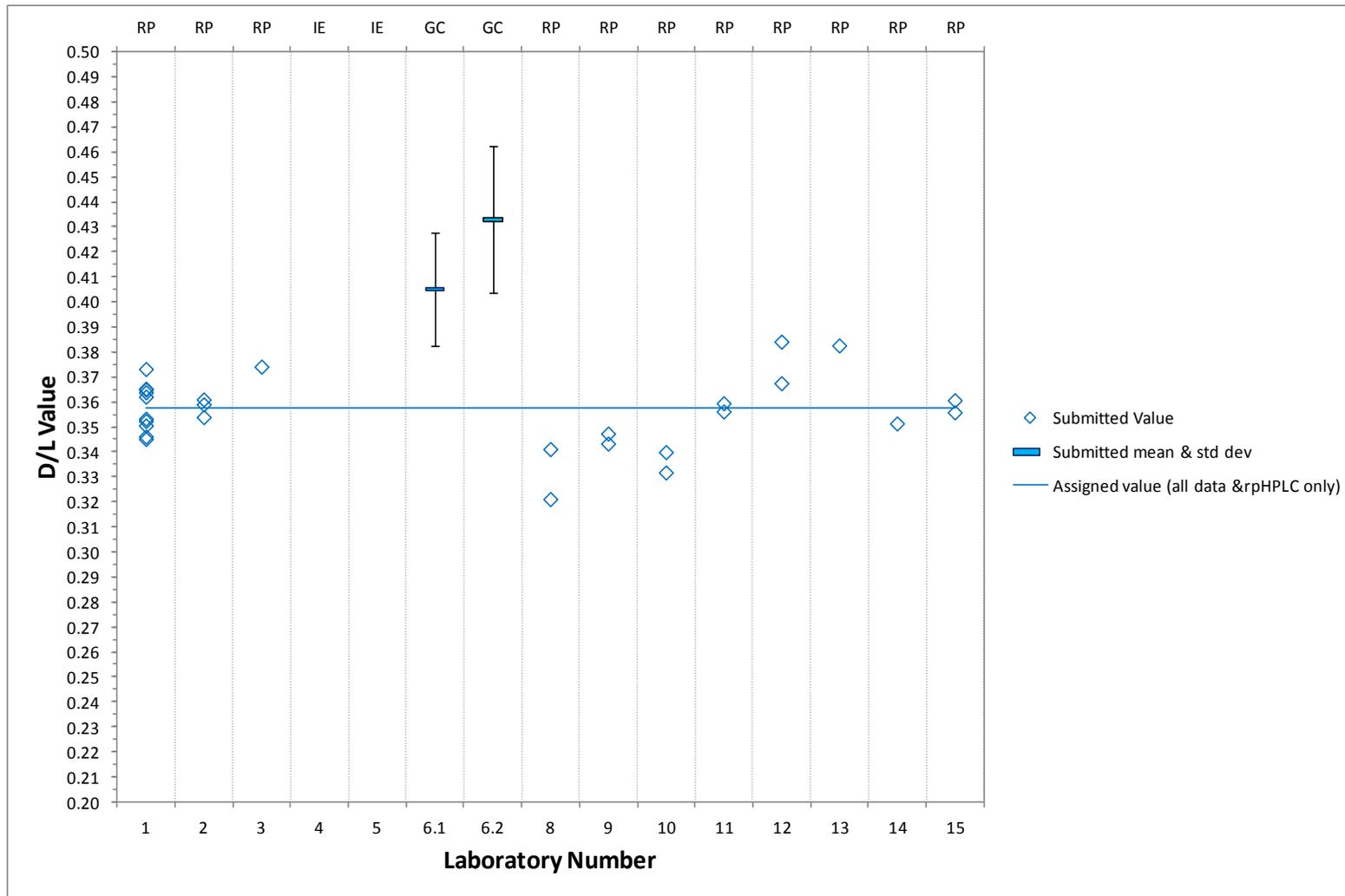


Figure 4.15: Experimental Expanded Uncertainty ($k=2$) of the Mean D/L value for Alanine (value of n displayed).

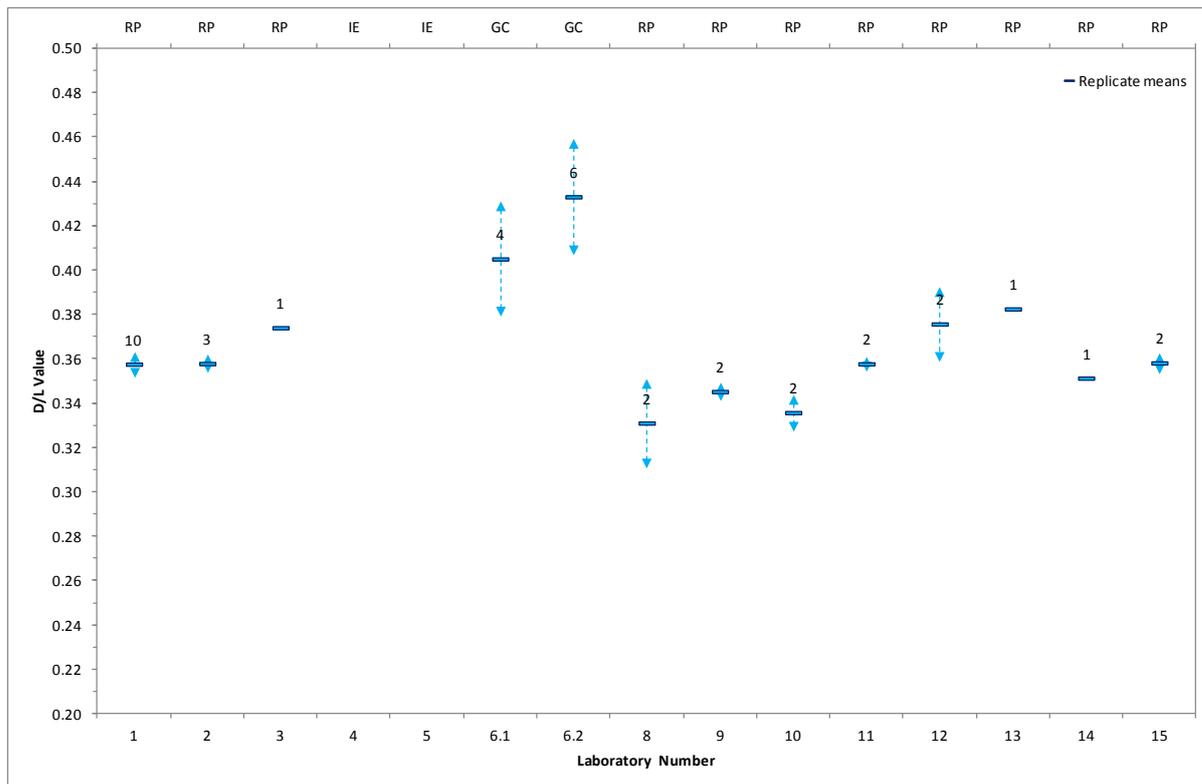


Figure 4.16: Experimental Expanded Uncertainty ($k=t_{(0.05,df)}$) of the Mean D/L value for Alanine (value of n displayed).

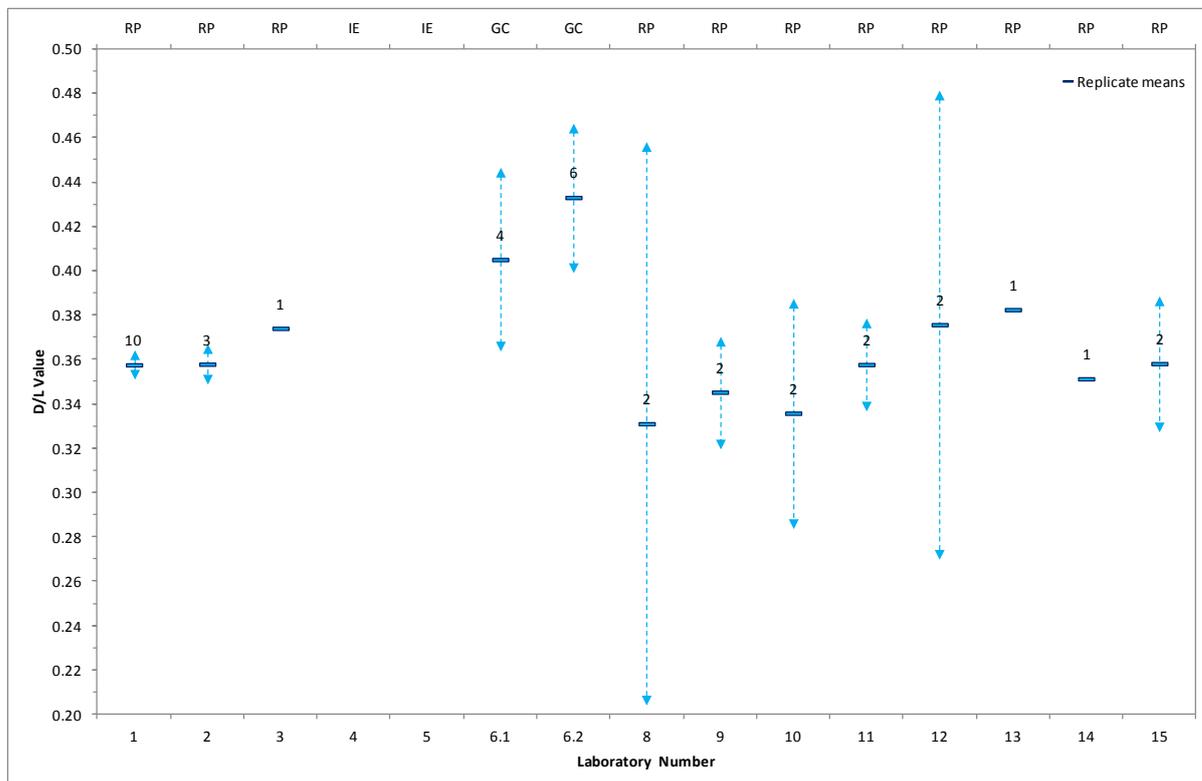


Table 4.17: Summary Statistics for L and D Valine Peak Area / Height Data

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)
1	RP	5193	5214	5048	5259	5415	5495	5577	5542	5639	5667	5405	10	213.5	3.95	67.5	1.25	2.50	2.262	2.83
2	RP	563	2595	2533								1897	3	1156.1	60.94	667.5	35.18	70.36	4.303	151.37
3	RP	2846										2846	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	6216	5950									6083	2	188.4	3.10	133.2	2.19	4.38	12.710	27.84
9	RP	2695	2710									2702	2	10.3	0.38	7.3	0.27	0.54	12.710	3.41
10	RP	1467	1524									1496	2	40.5	2.71	28.7	1.92	3.83	12.710	24.35
11	RP	2705	2800									2752	2	66.9	2.43	47.3	1.72	3.44	12.710	21.84
12	RP	4427	4316									4372	2	78.2	1.79	55.3	1.26	2.53	12.710	16.07
13	RP	6052										6052	1							
14	RP	2474										2474	1							
15	RP	2576	2595									2586	2	13.9	0.54	9.8	0.38	0.76	12.710	4.83
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	662	780	820	868	838	878	896	908	941	959	855	10	86.9	10.17	27.5	3.22	6.43	2.262	7.27
2	RP	100	461	454								338	3	206.2	60.93	119.1	35.18	70.36	4.303	151.37
3	RP	413										413	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	1024	1034									1029	2	7.1	0.69	5.0	0.49	0.98	12.710	6.23
9	RP	478	502									490	2	16.9	3.46	12.0	2.45	4.89	12.710	31.08
10	RP	295	293									294	2	1.4	0.48	1.0	0.34	0.68	12.710	4.30
11	RP	476	491									484	2	10.8	2.23	7.6	1.57	3.15	12.710	20.01
12	RP	902	830									866	2	51.1	5.90	36.2	4.17	8.35	12.710	53.06
13	RP	1273										1273	1							
14	RP	573										573	1							
15	RP	431	440									435	2	6.4	1.48	4.6	1.05	2.09	12.710	13.31
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	12.017	11.935									11.976	2	0.0580	0.48	0.0410	0.34	0.68	12.710	4.35
005	IE	10.242	11.562									10.902	2	0.9334	8.56	0.6600	6.05	12.11	12.710	76.95

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)
1	RP	314	313	297	311	311	318	312	319	318	318	313	10	6.5	2.06	2.0	0.65	1.30	2.262	1.47
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	345	328									337	2	12.2	3.63	8.6	2.57	5.13	12.710	32.62
9	RP	304	317									311	2	8.9	2.88	6.3	2.04	4.07	12.710	25.88
10	RP	311	304									308	2	5.1	1.65	3.6	1.17	2.33	12.710	14.83
11	RP	282	285									283	2	2.2	0.76	1.5	0.54	1.08	12.710	6.87
12	RP	246	260									253	2	10.2	4.01	7.2	2.84	5.67	12.710	36.05
13	RP	196										196	1							
14	RP	273										273	1							
15	RP	286	281									284	2	3.4	1.21	2.4	0.86	1.71	12.710	10.90
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	40	47	48	51	48	51	50	52	53	54	49	10	4.0	8.15	1.3	2.58	5.16	2.262	5.83
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	57	57									57	2	0.1	0.16	0.1	0.11	0.23	12.710	1.45
9	RP	49	53									51	2	3.0	5.96	2.1	4.21	8.42	12.710	53.53
10	RP	56	53									55	2	2.6	4.84	1.9	3.42	6.84	12.710	43.47
11	RP	45	45									45	2	0.3	0.56	0.2	0.40	0.79	12.710	5.04
12	RP	45	45									45	2	0.0	0.11	0.0	0.08	0.15	12.710	0.96
13	RP	37										37	1							
14	RP	57										57	1							
15	RP	43	43									43	2	0.1	0.27	0.1	0.19	0.38	12.710	2.42

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)
1	RP	0.127	0.150	0.163	0.165	0.155	0.160	0.161	0.164	0.167	0.169	0.158	10	0.0122	7.71	0.0039	2.44	4.88	2.262	5.52
2	RP	0.178	0.178	0.179								0.178	3	0.0008	0.47	0.0005	0.27	0.55	4.303	1.18
3	RP	0.145										0.145	1							
4	IE																			
5	IE																			
6.1 ¹	GC _A	0.126										0.126	12	0.0040	3.17	0.0012	0.92	1.83	2.201	2.02
6.2	GC																			
8	RP	0.165	0.174									0.170	2	0.0064	3.75	0.0045	2.65	5.31	12.710	33.74
9	RP	0.160	0.167									0.163	2	0.0050	3.08	0.0036	2.18	4.35	12.710	27.67
10	RP	0.181	0.173									0.177	2	0.0056	3.19	0.0040	2.25	4.51	12.710	28.65
11	RP	0.159	0.158									0.158	2	0.0003	0.20	0.0002	0.14	0.29	12.710	1.83
12	RP	0.184	0.173									0.178	2	0.0073	4.12	0.0052	2.91	5.82	12.710	37.01
13	RP	0.189										0.189	1							
14	RP	0.209										0.209	1							
15	RP	0.151	0.153									0.152	2	0.0014	0.94	0.0010	0.67	1.33	12.710	8.48

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

GC_A = derived using peak area

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

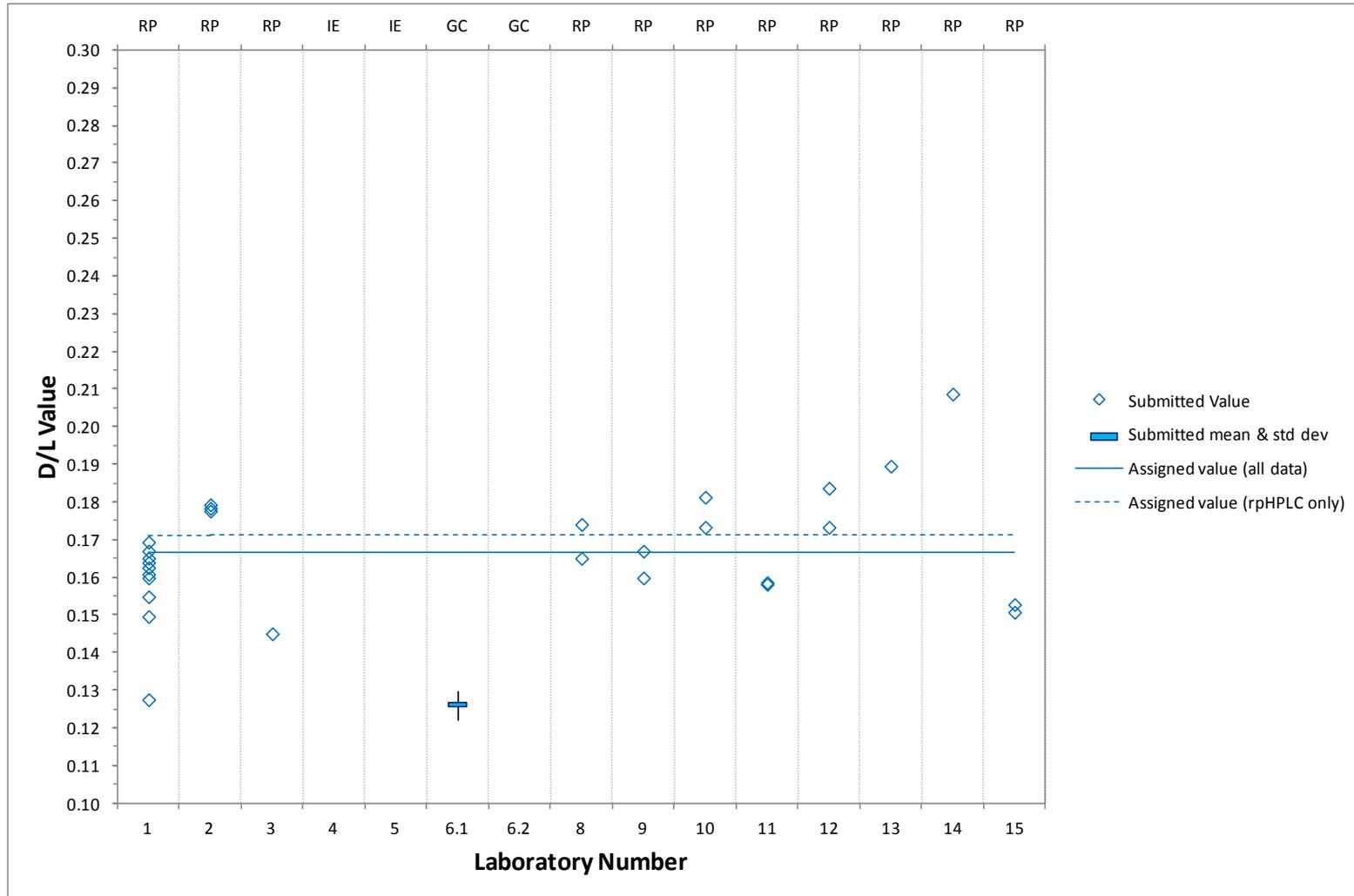


Figure 4.18: Experimental Expanded Uncertainty ($k=2$) of the Mean D/L value for **Valine** (value of n displayed).

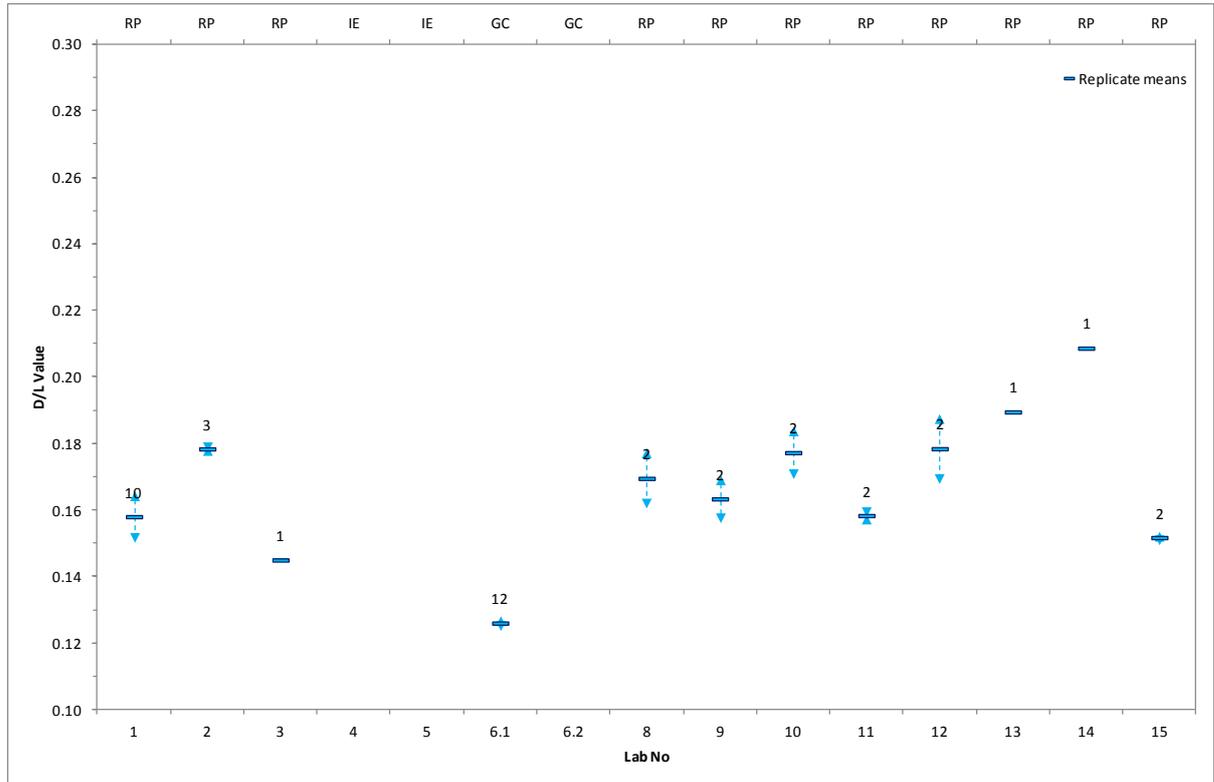


Figure 4.19: Experimental Expanded Uncertainty ($k=t_{(0.05,n)}$) of the Mean D/L value for **Valine** (value of n displayed).

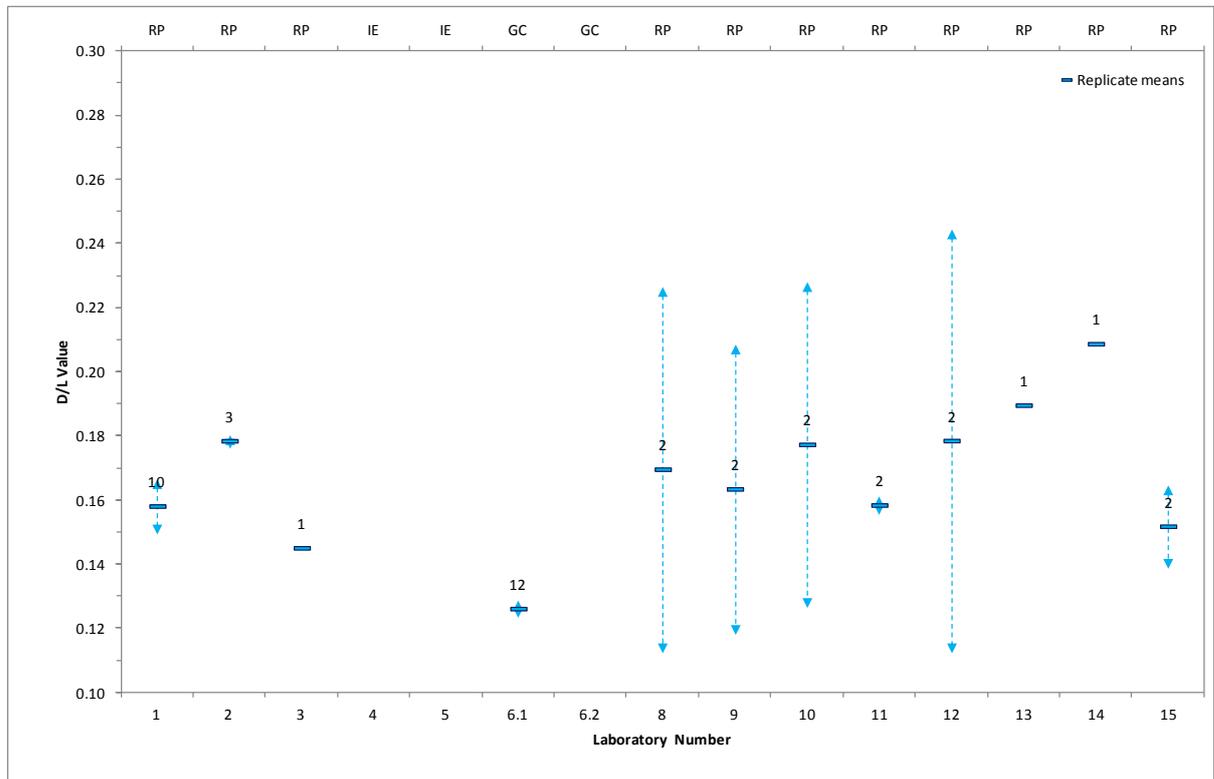


Table 4.20: Summary Statistics for L and D Phenylalanine Peak Area Data

4. STATISTICAL EVALUATION – Summary Statistics

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
		L-Phe peak area										mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% (k=t _{crit})
1	RP	3119	3092	3152	3190	3239	3221	9749	3262	3325	3329	3868	10	2068.0	53.47	654.0	16.91	33.82	2.262	38.25
2	RP	348	1569	1608								1175	3	716.3	60.96	413.6	35.19	70.39	4.303	151.43
3	RP	1836										1836	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	2967	2782									2875	2	131.0	4.56	92.6	3.22	6.44	12.710	40.96
9	RP	1414	1429									1422	2	10.4	0.73	7.4	0.52	1.03	12.710	6.57
10	RP	795	819									807	2	16.6	2.06	11.8	1.46	2.92	12.710	18.54
11	RP	1482	1551									1517	2	49.2	3.24	34.8	2.29	4.59	12.710	29.14
12	RP	2461	2425									2443	2	26.0	1.06	18.4	0.75	1.50	12.710	9.55
13	RP	4263										4263	1							
14	RP	1385										1385	1							
15	RP	1390	1404									1397	2	9.7	0.69	6.8	0.49	0.98	12.710	6.21
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	691	733	687	714	632	603	1536	945	604	574	772	10	288.3	37.35	91.2	11.81	23.62	2.262	26.72
2	RP	84	406	399								296	3	183.8	62.01	106.1	35.80	71.61	4.303	154.05
3	RP	476										476	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	822	819									821	2	2.0	0.25	1.4	0.17	0.35	12.710	2.22
9	RP	361	360									361	2	0.6	0.16	0.4	0.11	0.22	12.710	1.40
10	RP	201	205									203	2	2.7	1.34	1.9	0.95	1.90	12.710	12.04
11	RP	362	371									367	2	6.1	1.66	4.3	1.18	2.35	12.710	14.95
12	RP	632	617									625	2	10.3	1.65	7.3	1.17	2.34	12.710	14.86
13	RP	1083										1083	1							
14	RP	374										374	1							
15	RP	362	347									355	2	10.2	2.88	7.2	2.04	4.08	12.710	25.91

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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)
1	RP	188	186	186	189	186	187	545	188	188	187	223	10	113.2	50.79	35.8	16.06	32.12	2.262	36.33
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	165	153									159	2	8.1	5.09	5.7	3.60	7.20	12.710	45.74
9	RP	174	183									178	2	5.8	3.23	4.1	2.29	4.57	12.710	29.04
10	RP	184	178									181	2	4.2	2.30	2.9	1.62	3.25	12.710	20.65
11	RP	168	172									170	2	2.7	1.58	1.9	1.12	2.23	12.710	14.17
12	RP	149	160									154	2	7.3	4.74	5.2	3.35	6.70	12.710	42.57
13	RP	150										150	1							
14	RP	167										167	1							
15	RP	169	166									167	2	1.8	1.06	1.3	0.75	1.50	12.710	9.52
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	42	44	40	42	36	35	86	54	34	32	45	10	15.8	35.48	5.0	11.22	22.44	2.262	25.38
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	46	45									45	2	0.4	0.78	0.3	0.55	1.10	12.710	7.01
9	RP	45	46									45	2	1.1	2.34	0.8	1.66	3.32	12.710	21.07
10	RP	47	45									46	2	1.4	3.02	1.0	2.14	4.27	12.710	27.14
11	RP	41	41									41	2	0.0	0.00	0.0	0.00	0.00	12.710	0.02
12	RP	38	41									39	2	1.6	4.15	1.2	2.93	5.86	12.710	37.27
13	RP	38										38	1							
14	RP	45										45	1							
15	RP	44	41									42	2	2.0	4.63	1.4	3.28	6.55	12.710	41.63

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)
1	RP	0.221	0.237	0.218	0.224	0.195	0.187	0.158	0.181	0.182	0.172	0.198	10	0.0260	13.18	0.0082	4.17	8.33	2.262	9.43
2	RP	0.242	0.259	0.248								0.250	3	0.0085	3.42	0.0049	1.97	3.95	4.303	8.49
3	RP	0.259										0.259	1							
4	IE																			
5	IE																			
6.1 ¹	GC _A	0.228										0.228	14	0.0110	4.82	0.0029	1.29	2.58	2.160	2.79
6.2	GC																			
8	RP	0.277	0.294									0.286	2	0.0120	4.21	0.0085	2.98	5.95	12.710	37.84
9	RP	0.255	0.252									0.254	2	0.0023	0.89	0.0016	0.63	1.25	12.710	7.98
10	RP	0.253	0.251									0.252	2	0.0018	0.72	0.0013	0.51	1.02	12.710	6.49
11	RP	0.245	0.239									0.242	2	0.0038	1.58	0.0027	1.12	2.23	12.710	14.19
12	RP	0.257	0.255									0.256	2	0.0015	0.59	0.0011	0.42	0.83	12.710	5.30
13	RP	0.254										0.254	1							
14	RP	0.270										0.270	1							
15	RP	0.260	0.247									0.254	2	0.0091	3.57	0.0064	2.53	5.05	12.710	32.12

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

GC_A = derived using peak area

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

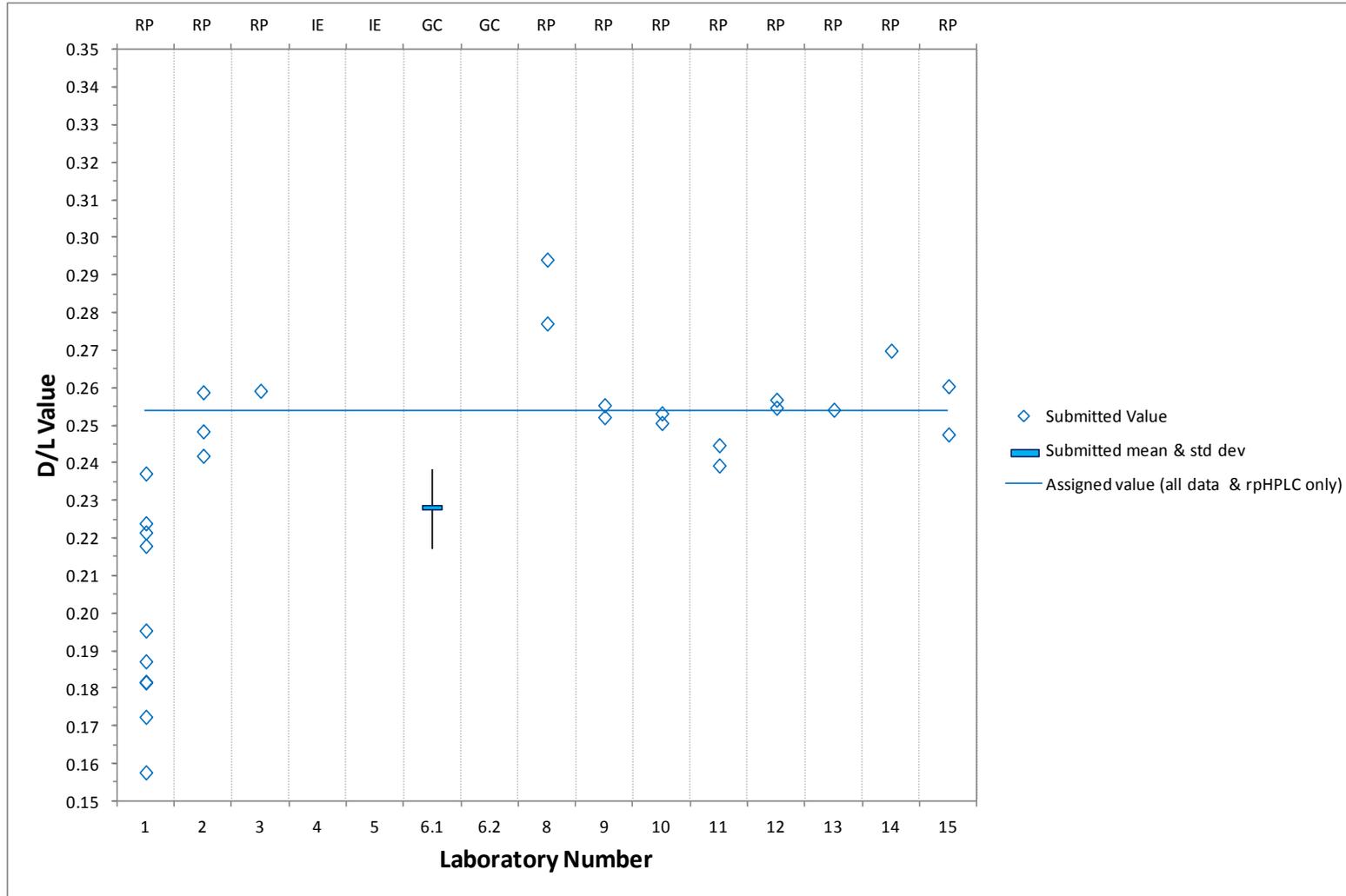


Figure 4.21: Experimental Expanded Uncertainty ($k=2$) of the Mean D/L value for Phenylalanine (value of n displayed).

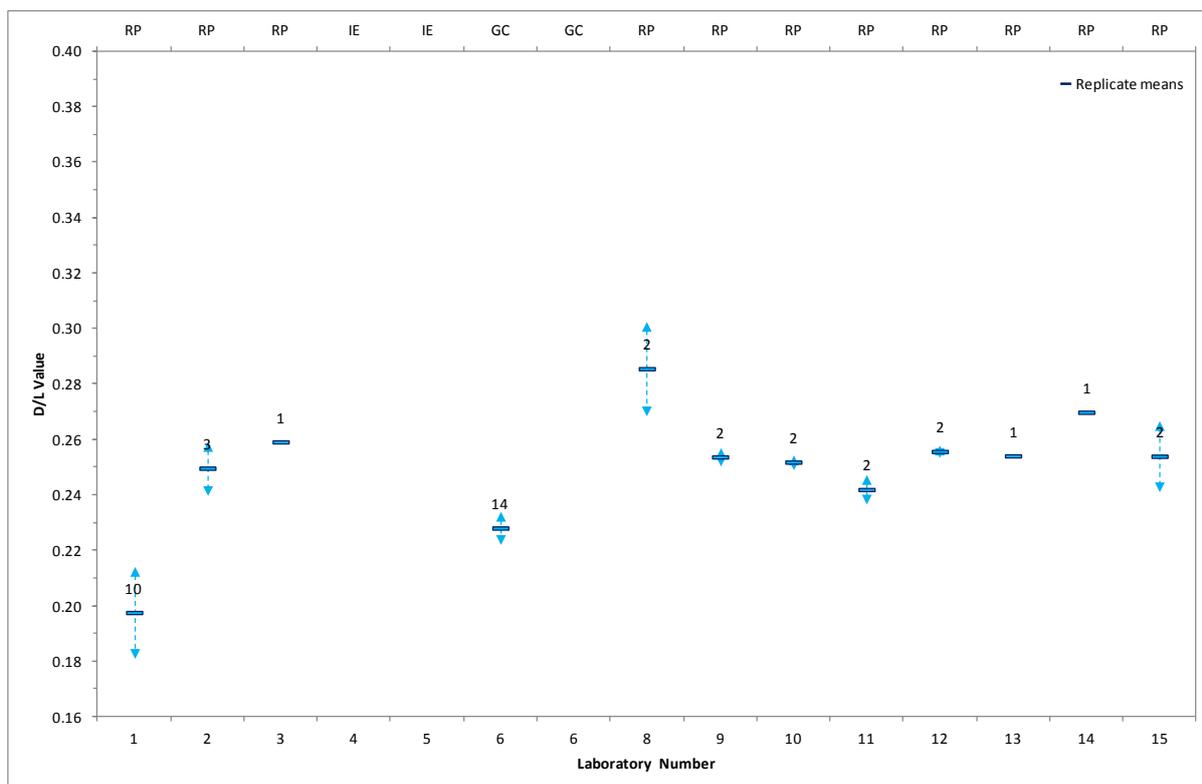


Figure 4.22: Experimental Expanded Uncertainty ($k=t_{(0.05,n)}$) of the Mean D/L value for Phenylalanine (value of n displayed).

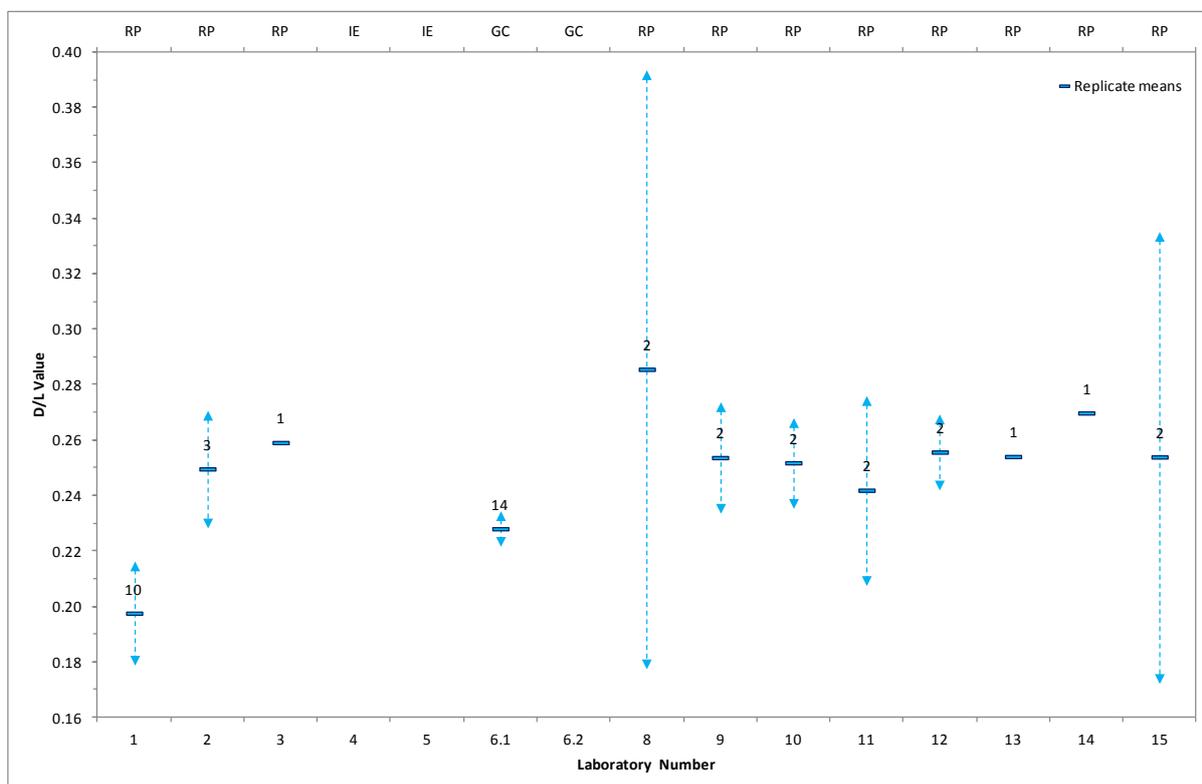


Table 4.23: Summary Statistics for D-Alloisoleucine/L-Isoleucine Peak Area Data

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	3346	3329	3291	3428	3468	3463	3567	3520	3595	3610	3462	10	113.3	3.27	35.8	1.04	2.07	2.262	2.34	
2	RP	335	1525	1574								1144	3	701.7	61.31	405.1	35.40	70.80	4.303	152.31	
3	RP	1698										1698	1								
4	IE*	2.099	2.112									2.106	2	0.0092	0.44	0.0065	0.31	0.62	12.710	3.92	
5	IE*	2.933	2.857									2.895	2	0.0537	1.86	0.0380	1.31	2.63	12.710	16.68	
6.1	GC																				
6.2	GC																				
8	RP	3550	3288									3419	2	185.1	5.41	130.9	3.83	7.66	12.710	48.66	
9	RP	1623	1648									1636	2	17.5	1.07	12.3	0.75	1.51	12.710	9.59	
10	RP	937	974									955	2	26.4	2.76	18.6	1.95	3.90	12.710	24.79	
11	RP	1699	1768									1734	2	48.5	2.80	34.3	1.98	3.96	12.710	25.16	
12	RP	2817	2763									2790	2	38.5	1.38	27.2	0.98	1.95	12.710	12.41	
13	RP	4085										4085	1								
14	RP	1698										1698	1								
15	RP	1579	1601									1590	2	15.7	0.99	11.1	0.70	1.39	12.710	8.86	
		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	441	431	395	429	444	449	406	442	454	429	432	10	18.7	4.34	5.9	1.37	2.74	2.262	3.10	
2	RP	70	335	332								245	3	151.9	61.92	87.7	35.75	71.49	4.303	153.81	
3	RP	420										420	1								
4	IE*	0.314	0.339									0.327	2	0.0177	5.41	0.0125	3.83	7.66	12.710	48.66	
5	IE*	0.445	0.435									0.440	2	0.0071	1.61	0.0050	1.14	2.27	12.710	14.44	
6.1	GC																				
6.2	GC																				
8	RP	572	574									573	2	1.4	0.24	1.0	0.17	0.33	12.710	2.13	
9	RP	327	466									397	2	98.1	24.73	69.4	17.49	34.98	12.710	222.30	
10	RP	174	200									187	2	18.2	9.72	12.8	6.88	13.75	12.710	87.39	
11	RP	407	477									442	2	49.6	11.21	35.1	7.93	15.86	12.710	100.78	
12	RP	563	719									641	2	110.8	17.28	78.3	12.22	24.44	12.710	155.30	
13	RP	1430										1430	1								
14	RP	539										539	1								
15	RP	341	281									311	2	42.6	13.70	30.1	9.69	19.38	12.710	123.13	

* = 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)
1	RP	202	200	194	203	199	201	199	203	203	202	201	10	2.8	1.40	0.9	0.44	0.88	2.262	1.00
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	197	181									189	2	11.3	5.95	8.0	4.20	8.41	12.710	53.43
9	RP	190	200									195	2	7.0	3.57	4.9	2.52	5.04	12.710	32.06
10	RP	206	202									204	2	3.3	1.60	2.3	1.13	2.26	12.710	14.39
11	RP	184	187									185	2	2.1	1.13	1.5	0.80	1.60	12.710	10.19
12	RP	162	173									168	2	7.4	4.42	5.2	3.12	6.25	12.710	39.72
13	RP	137										137	1							
14	RP	194										194	1							
15	RP	182	180									181	2	1.4	0.76	1.0	0.54	1.08	12.710	6.87
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	27	26	23	25	25	26	23	25	26	24	25	10	1.3	5.08	0.4	1.61	3.21	2.262	3.64
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	32	32									32	2	0.1	0.30	0.1	0.21	0.42	12.710	2.66
9	RP	38	57									47	2	12.9	27.15	9.1	19.20	38.40	12.710	244.02
10	RP	38	41									40	2	2.1	5.38	1.5	3.80	7.60	12.710	48.32
11	RP	44	50									47	2	4.5	9.56	3.2	6.76	13.52	12.710	85.89
12	RP	32	45									39	2	8.9	22.96	6.3	16.24	32.47	12.710	206.38
13	RP	48										48	1							
14	RP	62										62	1							
15	RP	39	32									35	2	5.5	15.43	3.9	10.91	21.82	12.710	138.69

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)
1	RP	0.132	0.129	0.120	0.125	0.128	0.130	0.114	0.125	0.126	0.119	0.125	10	0.0056	4.51	0.0018	1.43	2.85	2.262	3.23
2	RP	0.209	0.219	0.211								0.213	3	0.0055	2.60	0.0032	1.50	3.01	4.303	6.47
3	RP	0.247										0.247	1							
4	IE	0.149	0.161									0.155	2	0.0085	5.47	0.0060	3.87	7.74	12.710	49.20
5	IE	0.152	0.152									0.152	2	0.0000	0.00	0.0000	0.00	0.00	12.710	0.00
6.1 ¹	GC _A	0.168										0.168	14	0.0060	3.57	0.0016	0.95	1.91	2.160	2.06
6.2	GC																			
8	RP	0.161	0.175									0.168	2	0.0099	5.89	0.0070	4.17	8.33	12.710	52.96
9	RP	0.202	0.283									0.242	2	0.0574	23.70	0.0406	16.76	33.52	12.710	212.99
10	RP	0.186	0.205									0.195	2	0.0136	6.97	0.0096	4.93	9.86	12.710	62.68
11	RP	0.240	0.270									0.255	2	0.0215	8.43	0.0152	5.96	11.92	12.710	75.74
12	RP	0.200	0.260									0.230	2	0.0429	18.64	0.0303	13.18	26.36	12.710	167.51
13	RP	0.350										0.350	1							
14	RP	0.317										0.317	1							
15	RP	0.216	0.175									0.195	2	0.0287	14.68	0.0203	10.38	20.76	12.710	131.90

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

GC_A = derived using peak area

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

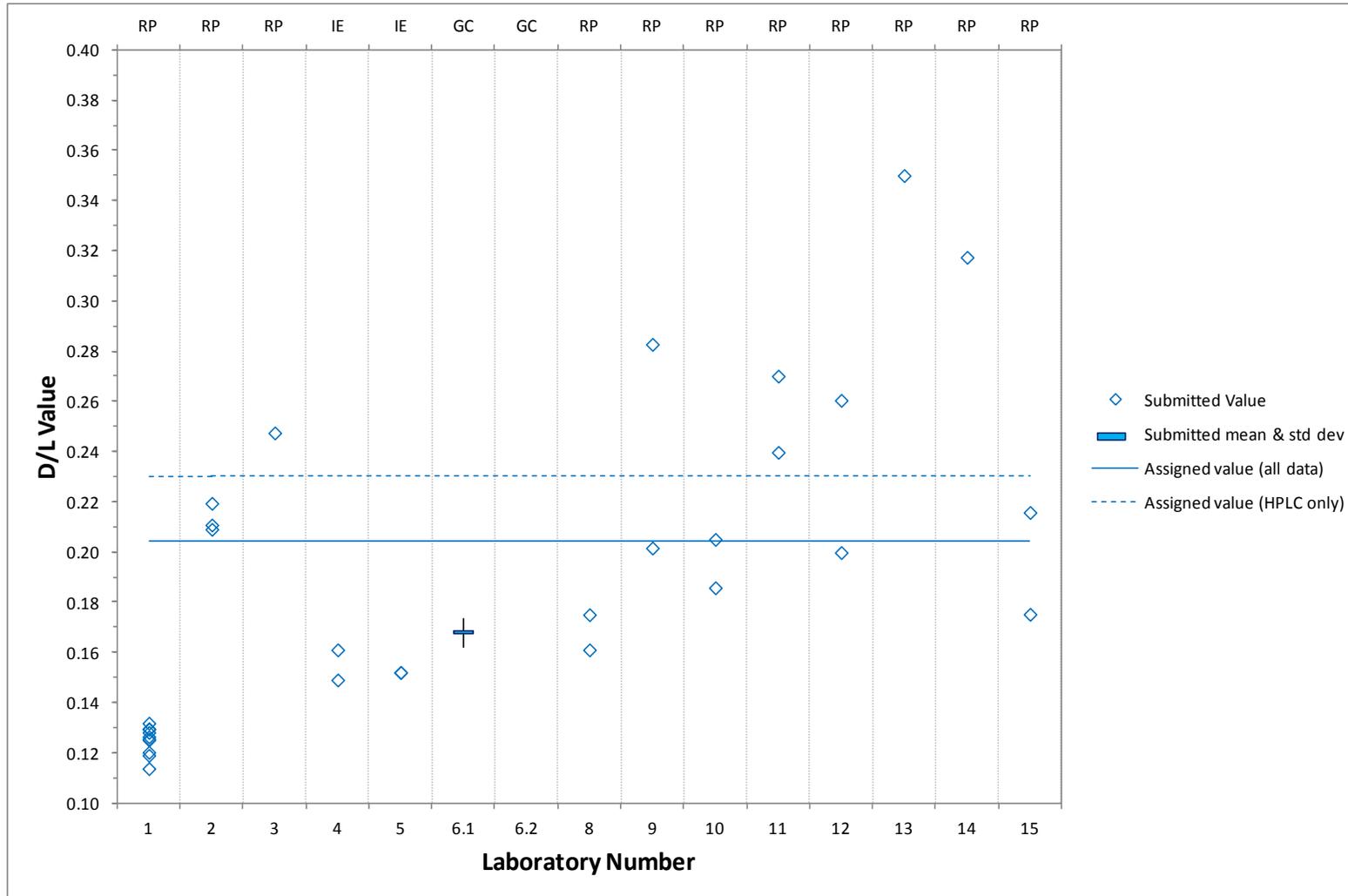


Figure 4.24: Experimental Expanded Uncertainty ($k=2$) of the Mean D/L value for D-Alloisoleucine/L-Isoleucine (value of n displayed).

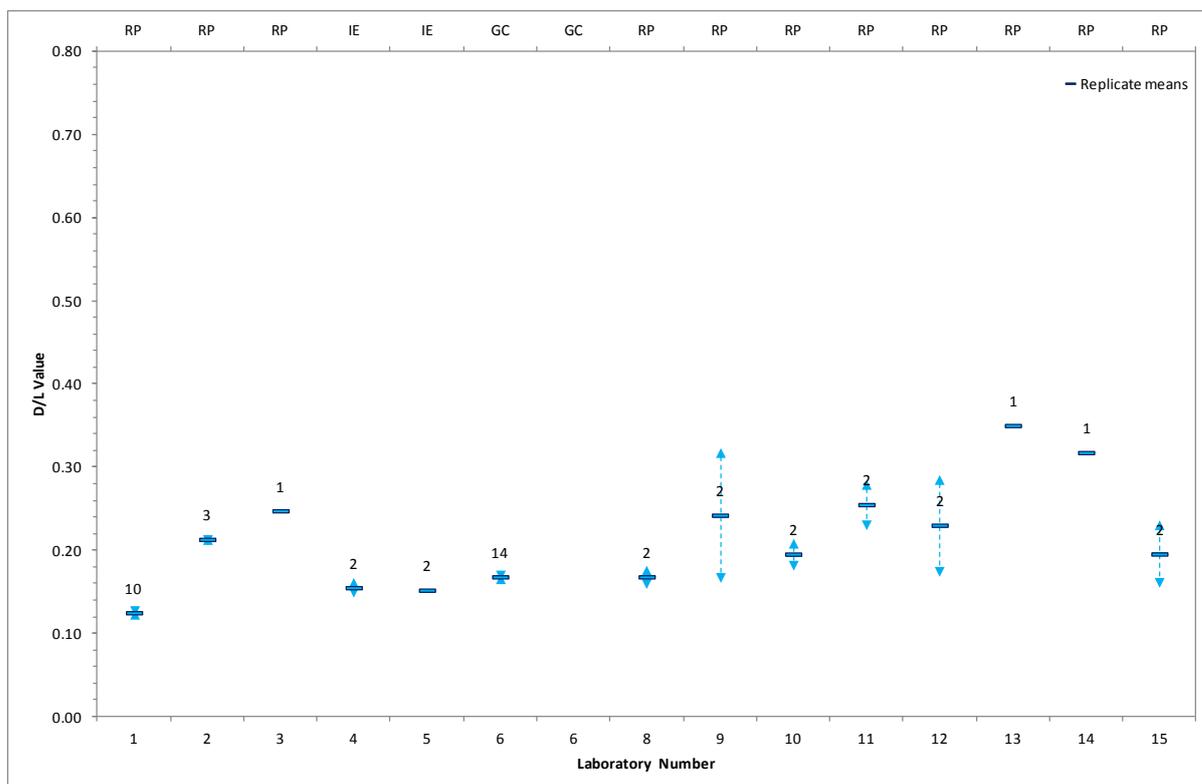


Figure 4.25: Experimental Expanded Uncertainty ($k=t_{(0.05,df)}$) of the Mean D/L value for D-Alloisoleucine/L-Isoleucine (value of n displayed).

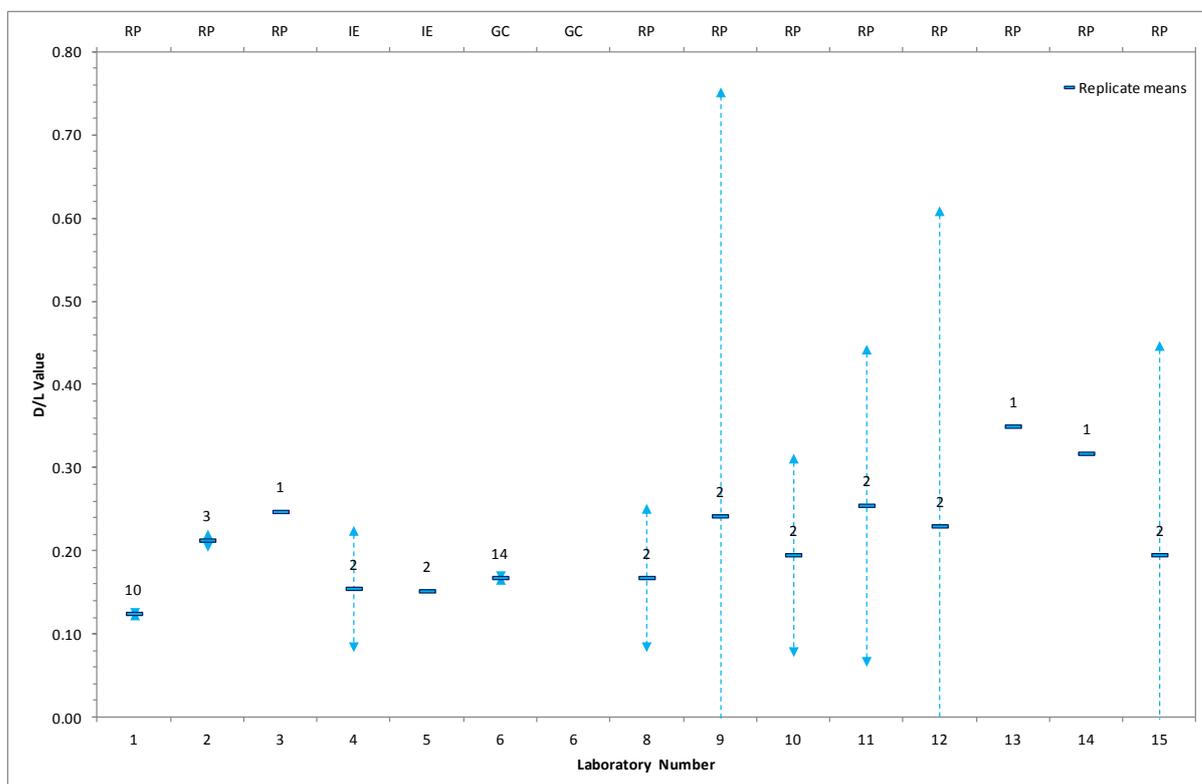


Table 4.26: Summary Statistics for L and D Leucine Peak Area Data

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)
1	RP	3091	3048	3177	3150	3148	3173	3252	3132	3241	3265	3168	10	70.1	2.21	22.2	0.70	1.40	2.262	1.58
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	3303	2976									3139	2	231.4	7.37	163.7	5.21	10.43	12.710	66.26
9	RP	1242	1262									1252	2	13.6	1.09	9.6	0.77	1.54	12.710	9.79
10	RP	707	729									718	2	15.5	2.16	11.0	1.53	3.05	12.710	19.41
11	RP	1365	1406									1385	2	28.5	2.06	20.1	1.45	2.91	12.710	18.47
12	RP	2131	2114									2123	2	12.2	0.57	8.6	0.41	0.81	12.710	5.15
13	RP	3584										3584	1							
14	RP	1404										1404	1							
15	RP	1240	1213									1227	2	19.5	1.59	13.8	1.12	2.24	12.710	14.26
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	806	733	664	754	764	819	749	869	1009	997	816	10	112.7	13.80	35.6	4.36	8.73	2.262	9.87
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	654	646									650	2	6.0	0.92	4.2	0.65	1.30	12.710	8.25
9	RP	277	274									276	2	2.3	0.82	1.6	0.58	1.16	12.710	7.36
10	RP	224	157									190	2	47.1	24.78	33.3	17.52	35.05	12.710	222.72
11	RP	435	424									429	2	7.5	1.74	5.3	1.23	2.46	12.710	15.66
12	RP	477	456									466	2	14.7	3.15	10.4	2.22	4.45	12.710	28.28
13	RP																			
14	RP																			
15	RP	255	225									240	2	20.6	8.59	14.6	6.07	12.14	12.710	77.16
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	2.040	2.046									2.043	2	0.0042	0.21	0.0030	0.15	0.29	12.710	1.87
005	IE	2.833	2.753									2.793	2	0.0566	2.03	0.0400	1.43	2.86	12.710	18.20

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										mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% (k=t _{crit})
1	RP	187	183	187	186	181	184	182	180	183	183	184	10	2.5	1.35	0.8	0.43	0.85	2.262	0.96
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	184	164									174	2	13.7	7.90	9.7	5.59	11.18	12.710	71.03
9	RP	195	205									200	2	7.2	3.59	5.1	2.54	5.08	12.710	32.26
10	RP	209	202									206	2	4.5	2.20	3.2	1.56	3.11	12.710	19.77
11	RP	198	199									198	2	0.8	0.39	0.5	0.28	0.55	12.710	3.50
12	RP	165	177									171	2	8.9	5.23	6.3	3.69	7.39	12.710	46.96
13	RP	161										161	1							
14	RP	215										215	1							
15	RP	192	183									187	2	6.2	3.34	4.4	2.36	4.72	12.710	29.98
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	49	44	39	45	44	47	42	50	57	56	47	10	5.8	12.31	1.8	3.89	7.79	2.262	8.81
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP	36	36									36	2	0.5	1.45	0.4	1.03	2.05	12.710	13.04
9	RP	44	45									44	2	0.7	1.68	0.5	1.19	2.38	12.710	15.11
10	RP	66	44									55	2	15.9	28.98	11.2	20.49	40.99	12.710	260.49
11	RP	63	60									61	2	2.1	3.41	1.5	2.41	4.82	12.710	30.62
12	RP	37	38									38	2	1.0	2.65	0.7	1.88	3.75	12.710	23.85
13	RP																			
14	RP																			
15	RP	39	34									37	2	3.8	10.33	2.7	7.30	14.61	12.710	92.82

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)
1	RP	0.261	0.241	0.209	0.239	0.243	0.258	0.230	0.277	0.311	0.305	0.257	10	0.0326	12.65	0.0103	4.00	8.00	2.262	9.05
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1 ¹	GC _A	0.187										0.187	14	0.0120	6.42	0.0032	1.72	3.43	2.160	3.71
6.2 ¹	GC _H	0.183										0.183	4	0.0060	3.28	0.0030	1.64	3.28	3.182	5.22
8	RP	0.198	0.217									0.208	2	0.0134	6.47	0.0095	4.58	9.16	12.710	58.19
9	RP	0.223	0.217									0.220	2	0.0042	1.91	0.0030	1.35	2.70	12.710	17.15
10	RP	0.316	0.215									0.266	2	0.0714	26.87	0.0505	19.00	38.00	12.710	241.49
11	RP	0.318	0.302									0.310	2	0.0118	3.80	0.0083	2.68	5.37	12.710	34.12
12	RP	0.224	0.216									0.220	2	0.0057	2.57	0.0040	1.82	3.64	12.710	23.12
13	RP																			
14	RP																			
15	RP	0.205	0.186									0.196	2	0.0137	7.00	0.0097	4.95	9.90	12.710	62.94

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

GC_A = derived using peak area

GC_H = derived using peak height

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

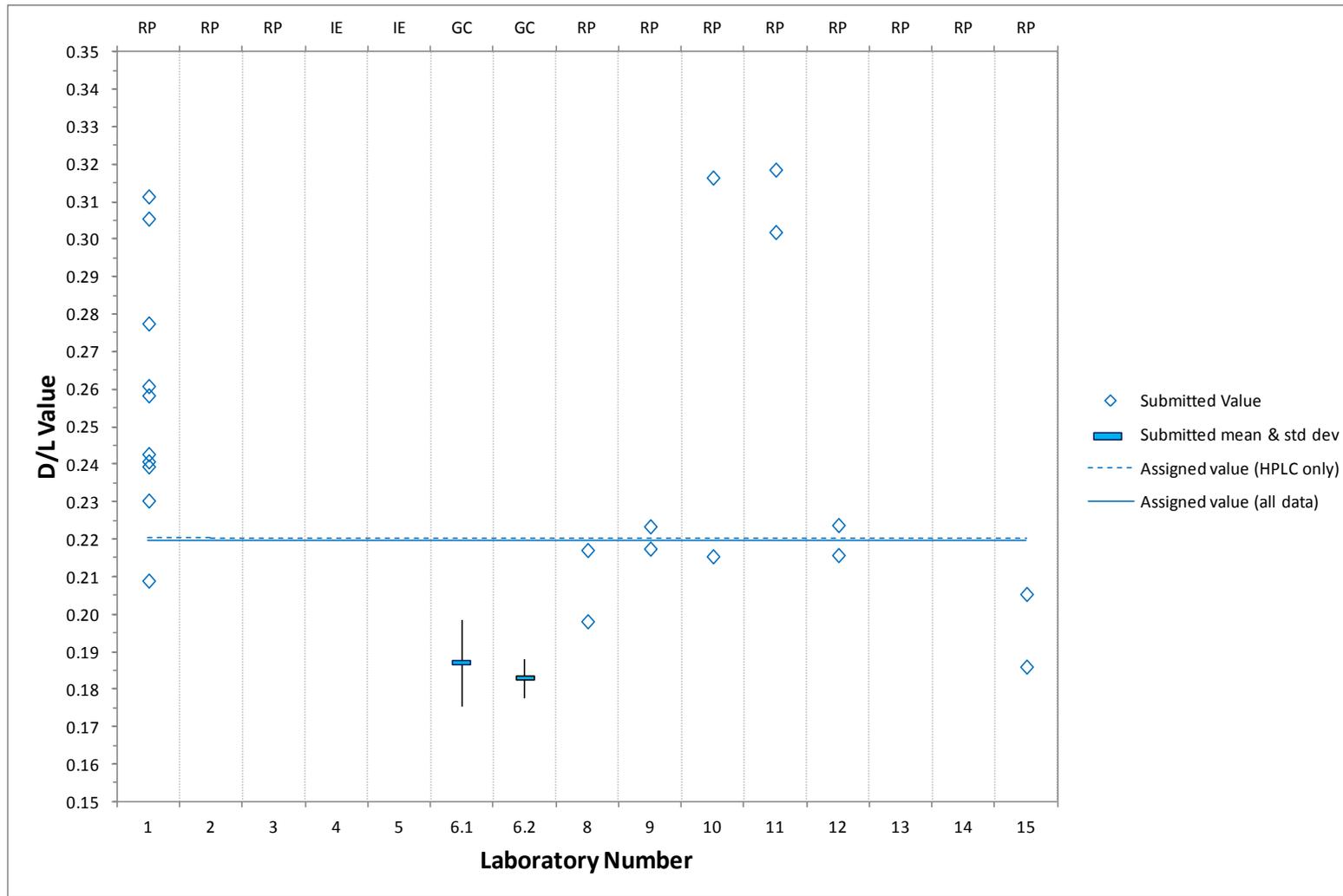


Figure 4.27: Experimental Expanded Uncertainty ($k=2$) of the Mean D/L value for **Leucine** (value of n displayed).

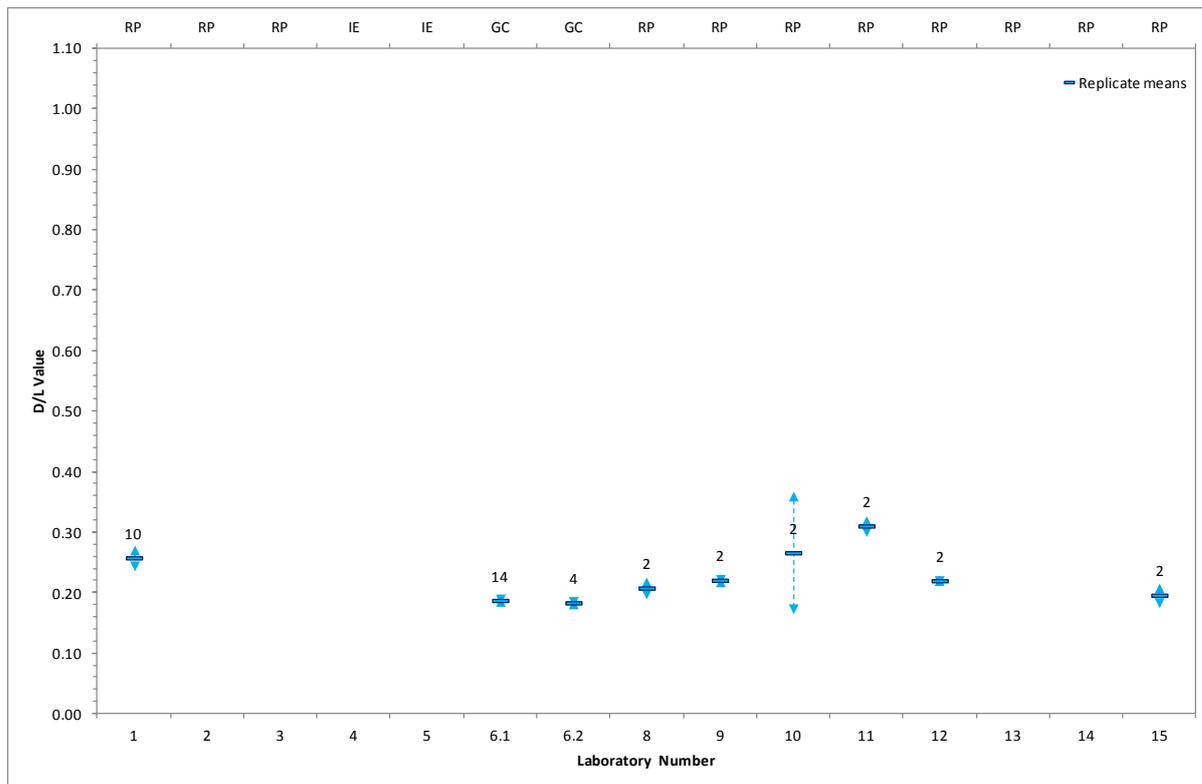


Figure 4.28: Experimental Expanded Uncertainty ($k=t_{(0.05,df)}$) of the Mean D/L value for **Leucine** (value of n displayed).

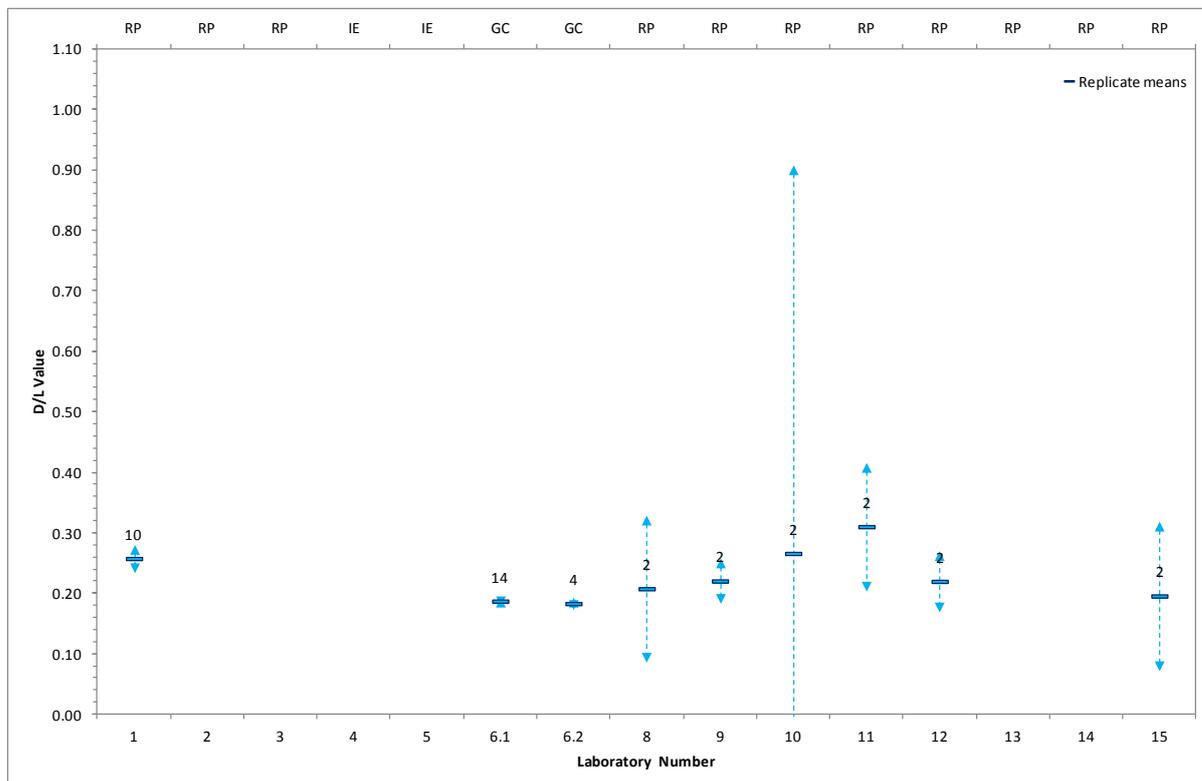


Table 4.29: Summary Statistics for L and D Tyrosine Peak Area Data

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)
1	RP																				
2	RP																				
3	RP																				
4	IE																				
5	IE																				
6.1	GC																				
6.2	GC																				
8	RP																				
9	RP	497	495									496	2	0.8	0.15	0.5	0.11	0.22	12.710	1.39	
10	RP	406	413									410	2	4.9	1.21	3.5	0.85	1.71	12.710	10.84	
11	RP	772	811									792	2	27.8	3.51	19.6	2.48	4.96	12.710	31.55	
12	RP	1546	1518									1532	2	20.3	1.33	14.4	0.94	1.87	12.710	11.91	
13	RP	1292										1292	1								
14	RP	321										321	1								
15	RP	428	415									421	2	9.4	2.22	6.6	1.57	3.15	12.710	19.99	
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																				
8	RP																				
9	RP	104	100									102	2	3.1	3.01	2.2	2.12	4.25	12.710	27.01	
10	RP	90	89									89	2	0.4	0.45	0.3	0.32	0.63	12.710	4.01	
11	RP	155										155	1								
12	RP	356	341									348	2	10.4	2.99	7.4	2.11	4.23	12.710	26.87	
13	RP																				
14	RP																				
15	RP	93	92									93	2	1.0	1.09	0.7	0.77	1.54	12.710	9.80	

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										mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% (k=t _{crit})
		a	b	c	d	e	f	g	h	i	j									
1	RP																			
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP																			
9	RP	50	52									51	2	1.2	2.35	0.8	1.66	3.32	12.710	21.08
10	RP	77	74									76	2	2.4	3.15	1.7	2.23	4.46	12.710	28.34
11	RP	72	74									73	2	1.3	1.85	1.0	1.30	2.61	12.710	16.58
12	RP	77	82									80	2	3.6	4.47	2.5	3.16	6.33	12.710	40.21
13	RP	37										37	1							
14	RP	32										32	1							
15	RP	43	40									41	2	1.6	3.97	1.2	2.81	5.62	12.710	35.71
		D-Tyr Conc										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																			
8	RP																			
9	RP	11	11									11	2	0.1	0.50	0.0	0.36	0.71	12.710	4.54
10	RP	17	16									17	2	0.8	4.80	0.6	3.40	6.79	12.710	43.18
11	RP	15										15	1							
12	RP	18	18									18	2	0.5	2.81	0.4	1.99	3.97	12.710	25.25
13	RP																			
14	RP																			
15	RP	9	9									9	2	0.3	2.84	0.2	2.01	4.02	12.710	25.53

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																				
8	RP																				
9	RP	0.210	0.202									0.206	2	0.0059	2.85	0.0042	2.02	4.03	12.710	25.62	
10	RP	0.221	0.216									0.218	2	0.0036	1.65	0.0025	1.17	2.34	12.710	14.85	
11	RP	0.201										0.201	1								
12	RP	0.230	0.225									0.227	2	0.0038	1.67	0.0027	1.18	2.36	12.710	14.97	
13	RP																				
14	RP																				
15	RP	0.218	0.222									0.220	2	0.0025	1.13	0.0018	0.80	1.60	12.710	10.19	

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

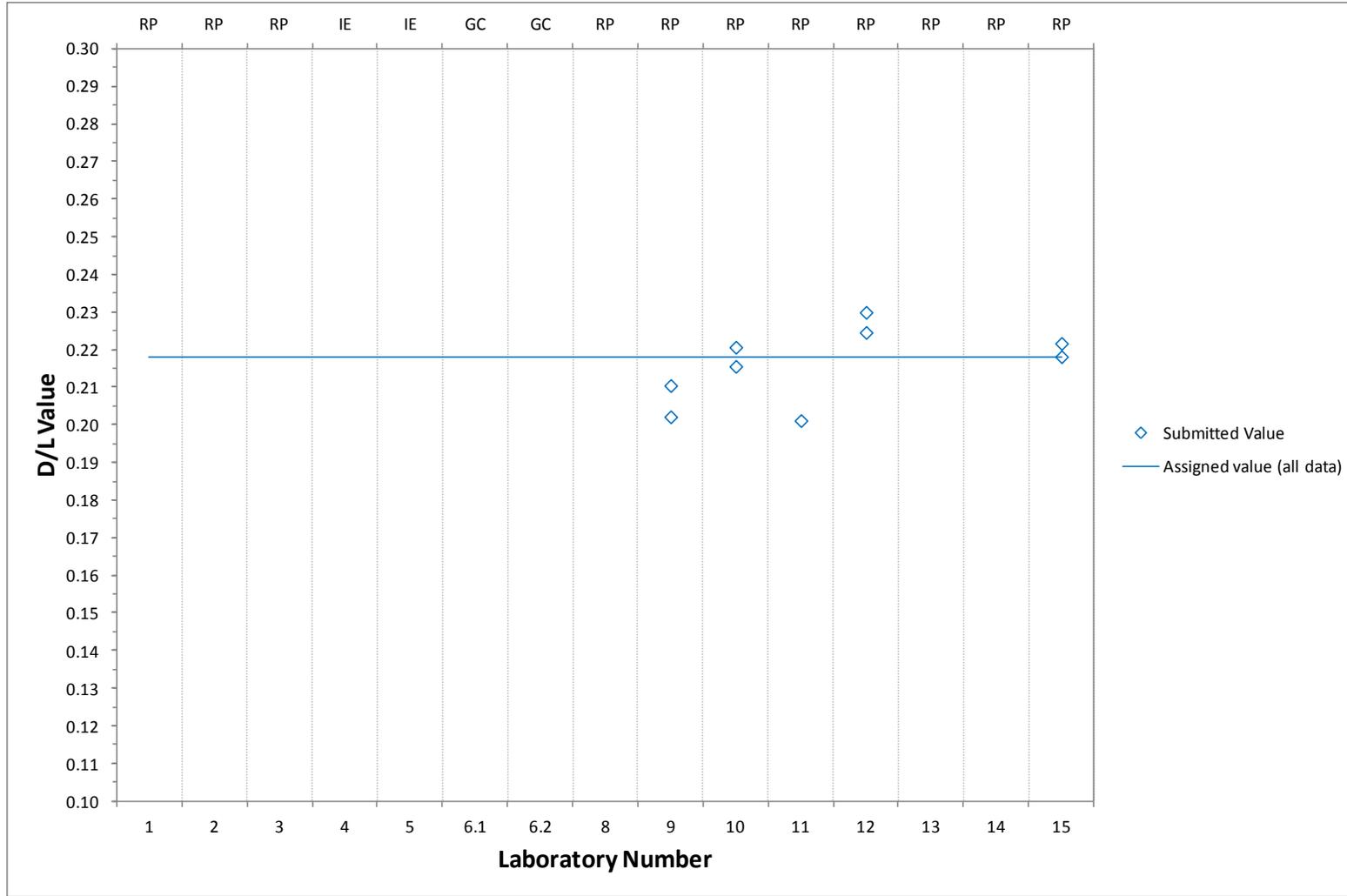


Figure 4.30: Experimental Expanded Uncertainty ($k=2$) of the Mean D/L value for Tyrosine (value of n displayed).

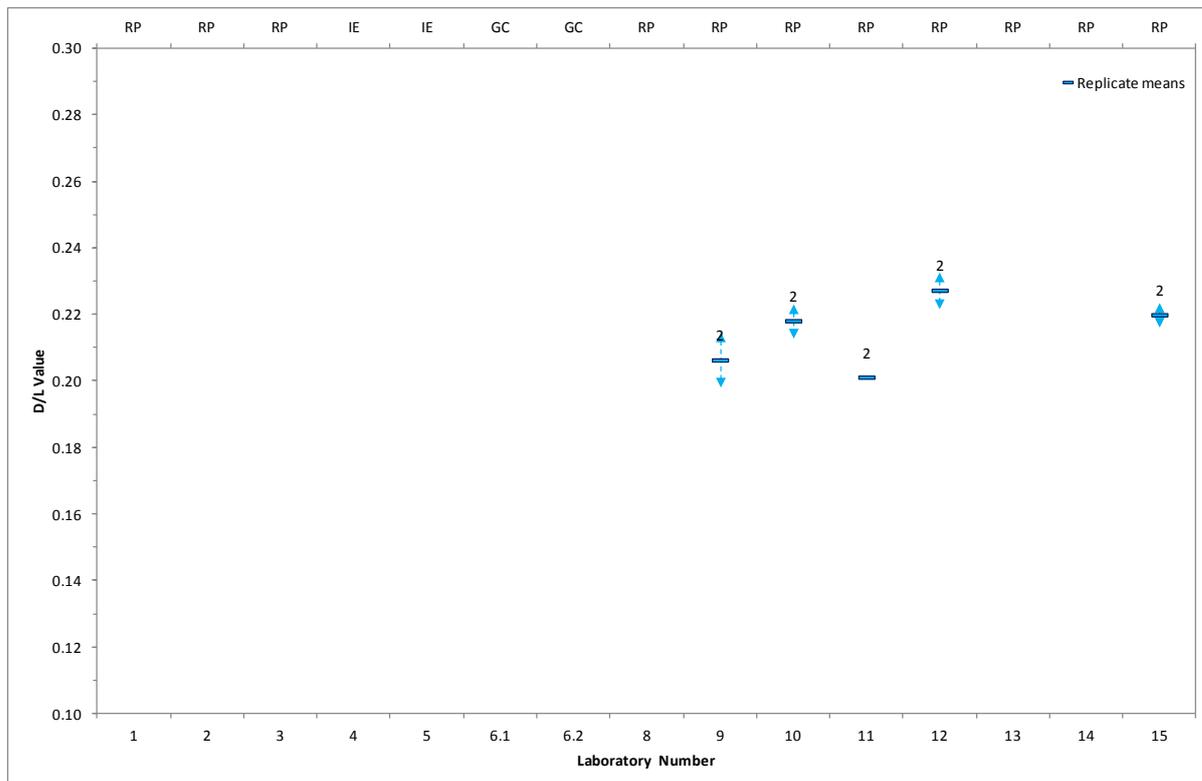


Figure 4.31: Experimental Expanded Uncertainty ($k=t_{(0.05,n)}$) of the Mean D/L value for Tyrosine (value of n displayed).

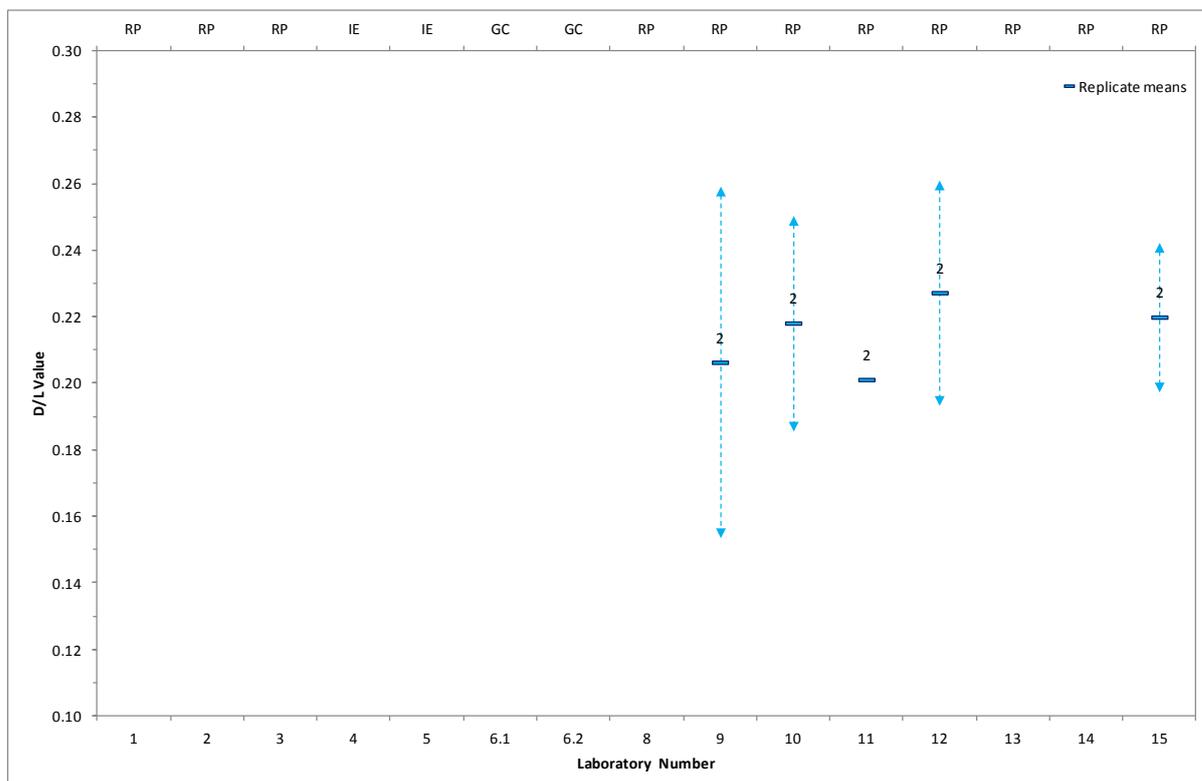


Table 4.32: Summary Statistics for L and D Methionine Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
		L-Met peak area										mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% (k=t _{crit})
		a	b	c	d	e	f	g	h	i	j									
1	RP																			
2	RP																			
3	RP																			
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP																			
9	RP	343	315									329	2	19.7	5.98	13.9	4.23	8.5	12.710	176.8
10	RP	123	121									122	2	2.0	1.65	1.4	1.17	2.3	12.710	18.1
11	RP	254	255									255	2	0.5	0.19	0.3	0.13	0.3	12.710	4.3
12	RP	362	346									354	2	11.2	3.17	7.9	2.24	4.5	12.710	100.7
13	RP	2331										2331	1							
14	RP	752										752	1							
15	RP	326	303									314	2	16.3	5.18	11.5	3.66	7.3	12.710	146.3
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																			
8	RP																			
9	RP	52	24									38	2	19.7	52.03	14.0	36.79	73.6	12.710	177.4
10	RP		14									14	1							
11	RP																			
12	RP																			
13	RP																			
14	RP																			
15	RP	39	39									39	2	0.3	0.66	0.2	0.47	0.9	12.710	2.3

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				
		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})
L-homoArginine peak area																				
1	RP	4968	4992	5094	5068	5230	5178	5368	5214	5313	5349	5178	10	143.5	2.77	45.4	0.88	1.75	2.262	1.98
2	RP	451	2057	2024								1511	3	918.0	60.77	530.0	35.08	70.17	4.303	150.95
3	RP	1597										1597	1							
4	IE																			
5	IE																			
6.1	GC																			
6.2	GC																			
8	RP																			
9	RP	1462	1412									1437	2	35.9	2.50	25.4	1.77	3.54	12.710	22.47
10	RP	778	827									803	2	35.0	4.36	24.7	3.08	6.16	12.710	39.17
11	RP	793	812									803	2	13.4	1.67	9.5	1.18	2.36	12.710	14.97
12	RP	1487	1370									1429	2	82.8	5.80	58.6	4.10	8.20	12.710	52.11
13	RP	2553										2553	1							
14	RP	749										749	1							
15	RP	744	762									753	2	13.2	1.75	9.3	1.24	2.47	12.710	15.73
Norleucine peak height																				
1	RP																			
2	RP																			
3	RP																			
4	IE	1.185	1.186									1	2	0.0	0.06	0.0	0.04	0.08	12.710	0.54
5	IE	1.673	1.634									2	2	0.0	1.67	0.0	1.18	2.36	12.710	14.99
6.1	GC																			
6.2	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;

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

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 Mollusc Shell (B) 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, only for alanine, does GC appear to contribute to a high tail and for valine and leucine, GC D/L values are the lowest. However, it is not possible to determine the significance of these differences from this study and histograms suggest that for this test material, GC results for these amino acids simply lay at the extreme of normal distributions from all the data. In this test material GC results for, aspartic acid / asparagine, glutamic acid/ glutamine, phenylalanine and alloisoleucine/isoleucine fall within the general distribution of the data. However, 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 Mollusc Shell (B) 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	0.404	assigned value	0.404	assigned value	0.209	assigned value	0.210
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
1	RP	0.372	-8.0	0.372	-7.9	0.157	-25.1	0.157	-25.5
2	RP	0.382	-5.4	0.382	-5.4	0.187	-10.4	0.187	-10.9
3	RP	0.382	-5.4	0.382	-5.4	0.186	-10.9	0.186	-11.3
4	IE								
5	IE								
6.1	GC	0.413	2.3			0.196	-6.3		
6.2	GC								
8	RP	0.404	0.0	0.404	0.1	0.208	-0.6	0.208	-1.1
9	RP	0.411	1.8	0.411	1.8	0.214	2.4	0.214	1.8
10	RP	0.411	1.7	0.411	1.7	0.216	3.5	0.216	2.9
11	RP	0.402	-0.4	0.402	-0.4	0.210	0.6	0.210	0.0
12	RP	0.412	2.0	0.412	2.1	0.215	2.9	0.215	2.4
13	RP	0.404	0.0	0.404	0.0	0.214	2.4	0.214	1.9
14	RP	0.403	-0.1	0.403	0.0	0.203	-2.8	0.203	-3.3
15	RP	0.407	0.8	0.407	0.8	0.218	4.0	0.218	3.5

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 Mollusc Shell (B) 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	0.436	assigned value	0.658	assigned value	0.358	assigned value	0.358
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
1	RP	0.319	-26.9			0.358	-0.1	0.358	0.0
2	RP	0.452	3.6	0.610	-7.4	0.358	0.0	0.358	0.0
3	RP	0.412	-5.5	0.707	7.4	0.374	4.5	0.374	4.6
4	IE								
5	IE								
6.1	GC					0.405	13.2		
6.2	GC					0.433	21.0		
8	RP	0.462	5.9			0.331	-7.5	0.331	-7.5
9	RP	0.454	4.1	0.784	19.1	0.345	-3.5	0.345	-3.5
10	RP	0.447	2.3	0.575	-12.7	0.336	-6.2	0.336	-6.1
11	RP	0.366	-16.1			0.358	0.0	0.358	0.0
12	RP	0.431	-1.3	0.757	14.9	0.376	5.0	0.376	5.0
13	RP	0.436	0.0	0.350	-46.9	0.382	6.9	0.382	6.9
14	RP	0.351	-19.6	0.833	26.5	0.351	-1.8	0.351	-1.8
15	RP	0.465	6.6	0.575	-12.7	0.358	0.1	0.358	0.1

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 Mollusc Shell (B) 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	0.166	assigned value	0.170	assigned value	0.254	assigned value	0.254
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
1	RP	0.158	-5.1	0.158	-6.8	0.198	-22.2	0.198	-22.2
2	RP	0.178	7.2	0.178	5.2	0.250	-1.7	0.250	-1.7
3	RP	0.145	-12.9	0.145	-14.5	0.259	2.1	0.259	2.0
4	IE								
5	IE								
6.1	GC	0.126	-24.3			0.228	-10.2		
6.2	GC								
8	RP	0.170	1.9	0.170	0.0	0.286	12.5	0.286	12.5
9	RP	0.163	-1.9	0.163	-3.6	0.254	0.0	0.254	-0.1
10	RP	0.177	6.5	0.177	4.6	0.252	-0.8	0.252	-0.8
11	RP	0.158	-4.9	0.158	-6.6	0.242	-4.7	0.242	-4.7
12	RP	0.178	7.2	0.178	5.3	0.256	0.7	0.256	0.7
13	RP	0.189	13.9	0.189	11.8	0.254	0.1	0.254	0.1
14	RP	0.209	25.4	0.209	23.1	0.270	6.3	0.270	6.3
15	RP	0.152	-8.8	0.152	-10.5	0.254	0.0	0.254	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 Mollusc Shell (B) 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	0.204	assigned value	0.230	assigned value	0.220	assigned value	0.220
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
1	RP	0.125	-38.9	0.125	-45.7	0.257	17.2	0.257	16.8
2	RP	0.213	4.3	0.213	-7.4				
3	RP	0.247	21.1	0.247	7.5				
4	IE	0.155	-24.1						
5	IE	0.152	-25.6						
6.1	GC	0.168	-17.7			0.187	-14.9		
6.2	GC					0.183	-16.7		
8	RP	0.168	-17.7	0.168	-27.0	0.208	-5.5	0.208	-5.8
9	RP	0.242	18.6	0.242	5.2	0.220	0.3	0.220	0.0
10	RP	0.195	-4.4	0.195	-15.1	0.266	21.0	0.266	20.6
11	RP	0.255	24.8	0.255	10.8	0.310	41.2	0.310	40.7
12	RP	0.230	12.7	0.230	0.0	0.220	0.0	0.220	-0.3
13	RP	0.350	71.4	0.350	52.1				
14	RP	0.317	55.4	0.317	38.0				
15	RP	0.195	-4.3	0.195	-15.1	0.196	-11.0	0.196	-11.2

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 Mollusc Shell (B) Test Material (continued)

Lab No.	method	Total Hydrolysed Amino Acid (THAA)	
		Tyr D/L (rpHPLC)	
		assigned value	0.218
		result D/L	relative bias %
1	RP		
2	RP		
3	RP		
4	IE		
5	IE		
6.1	GC		
6.2	GC		
8	RP		
9	RP	0.206	-5.4
10	RP	0.218	0.0
11	RP	0.201	-7.8
12	RP	0.227	4.2
13	RP		
14	RP		
15	RP	0.220	0.8

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)	12	0.404	0.0103	2.55	0.0030	0.74
Asx D/L (rpHPLC)	11	0.404	0.0102	2.52	0.0031	0.76
Glx D/L (all ^a)	12	0.209	0.0099	4.75	0.0029	1.37
Glx D/L (rpHPLC)	11	0.210	0.0090	4.30	0.0027	1.30
Ser D/L (rpHPLC)	11	0.436	0.0247	5.67	0.0075	1.71
Arg D/L (rpHPLC)	8	0.658	0.1349	20.49	0.0477	7.24
Ala D/L (all ^a)	13	0.358	0.0239	6.69	0.0066	1.86
Ala D/L (rpHPLC)	11	0.358	0.0185	5.18	0.0056	1.56
Val D/L (all ^a)	12	0.166	0.0177	10.66	0.0051	3.08
Val D/L (rpHPLC)	11	0.170	0.0166	9.78	0.0050	2.95
Phe D/L (all ^a)	12	0.254	0.0070	2.78	0.0020	0.80
Phe D/L (rpHPLC)	11	0.254	0.0064	2.51	0.0019	0.76
D-Aile/L-Ile (all ^b)	14	0.204	0.0601	29.43	0.0161	7.86
D-Aile/L-Ile (rpHPLC)	11	0.230	0.0514	22.32	0.0155	6.73
Leu D/L (all ^a)	9	0.220	0.0484	22.04	0.0161	7.35
Leu D/L (rpHPLC)	7	0.220	0.0367	16.66	0.0139	6.30
Tyr D/L (rpHPLC)	5	0.218	0.0136	6.22	0.0061	2.78

^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)	0.404	9	12	75%
Asx D/L (rpHPLC)	0.404	8	11	73%
Glx D/L (all ^a)	0.209	9	12	75%
Glx D/L (rpHPLC)	0.210	8	11	73%
Ser D/L (rpHPLC)	0.436	8	11	73%
Arg D/L (rpHPLC)	0.658	7	8	88%
Ala D/L (all ^a)	0.358	12	13	92%
Ala D/L (rpHPLC)	0.358	11	11	100%
Val D/L (all ^a)	0.166	10	12	83%
Val D/L (rpHPLC)	0.170	10	11	91%
Phe D/L (all ^a)	0.254	8	12	67%
Phe D/L (rpHPLC)	0.254	8	11	73%
D-Aile/L-Ile (all ^b)	0.204	13	14	93%
D-Aile/L-Ile (rpHPLC)	0.230	9	11	82%
Leu D/L (all ^a)	0.220	9	9	100%
Leu D/L (rpHPLC)	0.220	6	7	86%
Tyr D/L (rpHPLC)	0.218	5	5	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

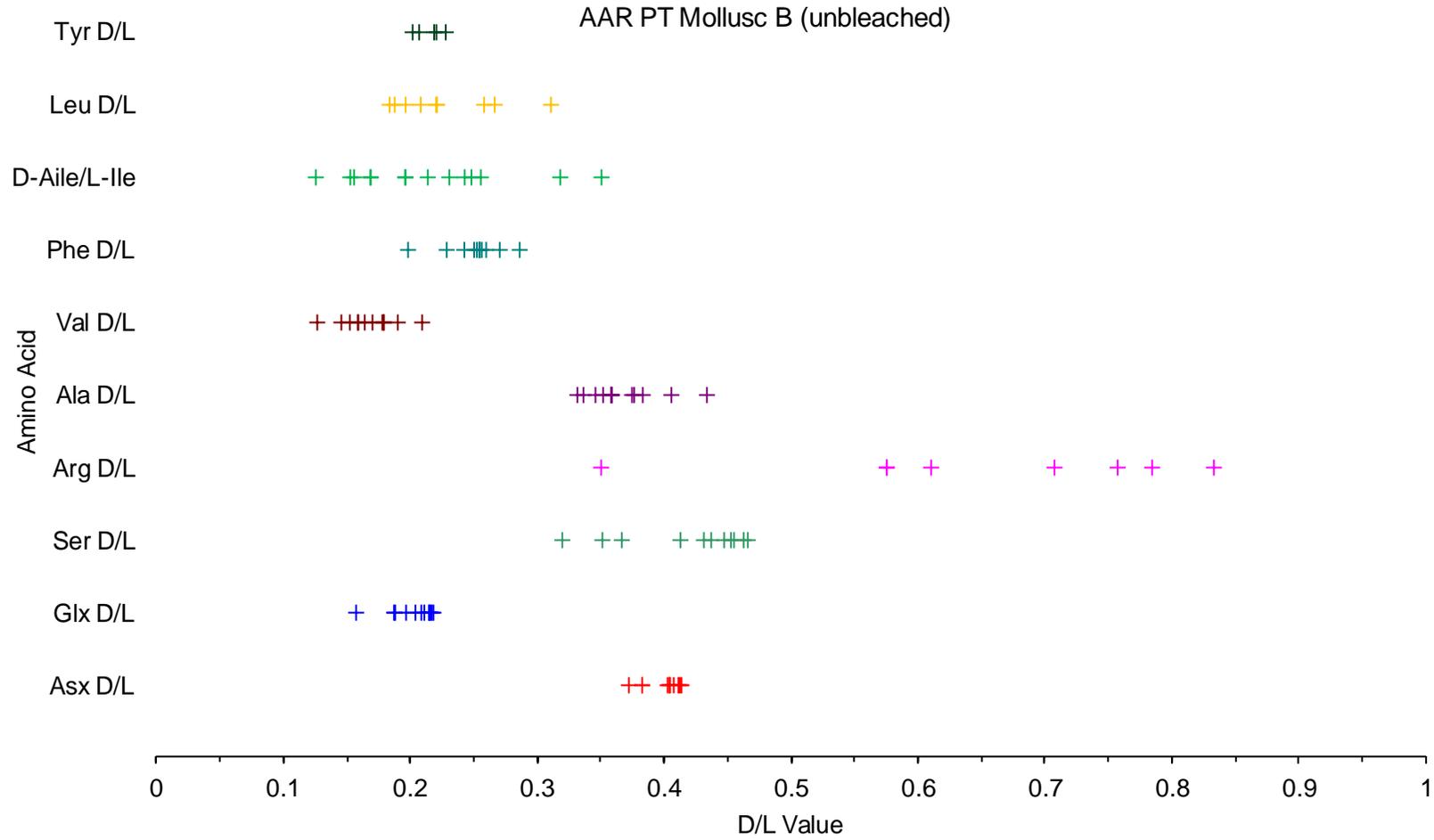


Figure 5.2: Relative Percentage Bias for **Aspartic Acid / Asparagine D/L Results (all data)** in Mollusc Shell (B) Test Material

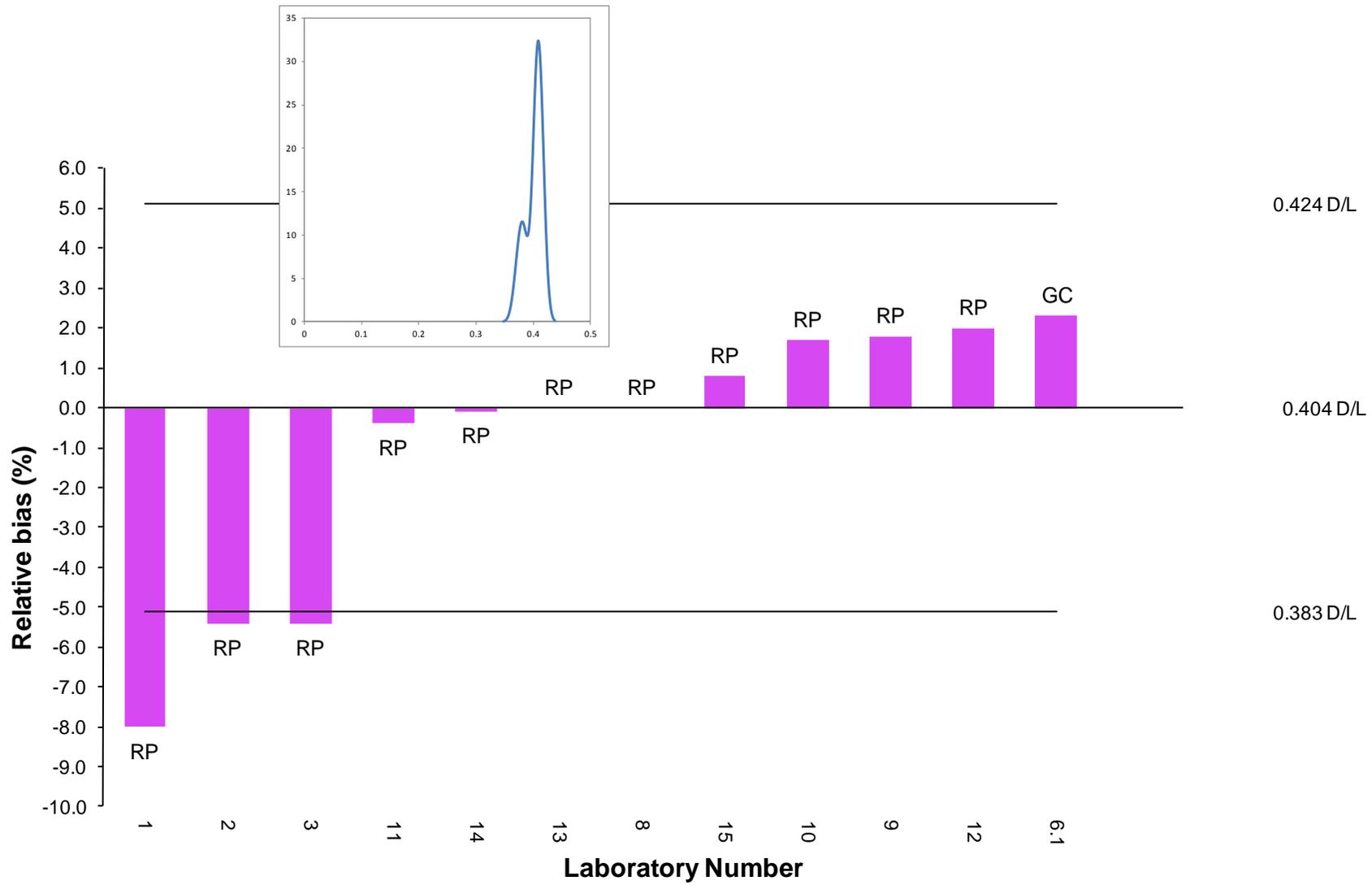


Figure 5.3: Relative Percentage Bias for **Aspartic Acid / Asparagine D/L Results (rpHPLC data only)** in Mollusc Shell (B) Test Material

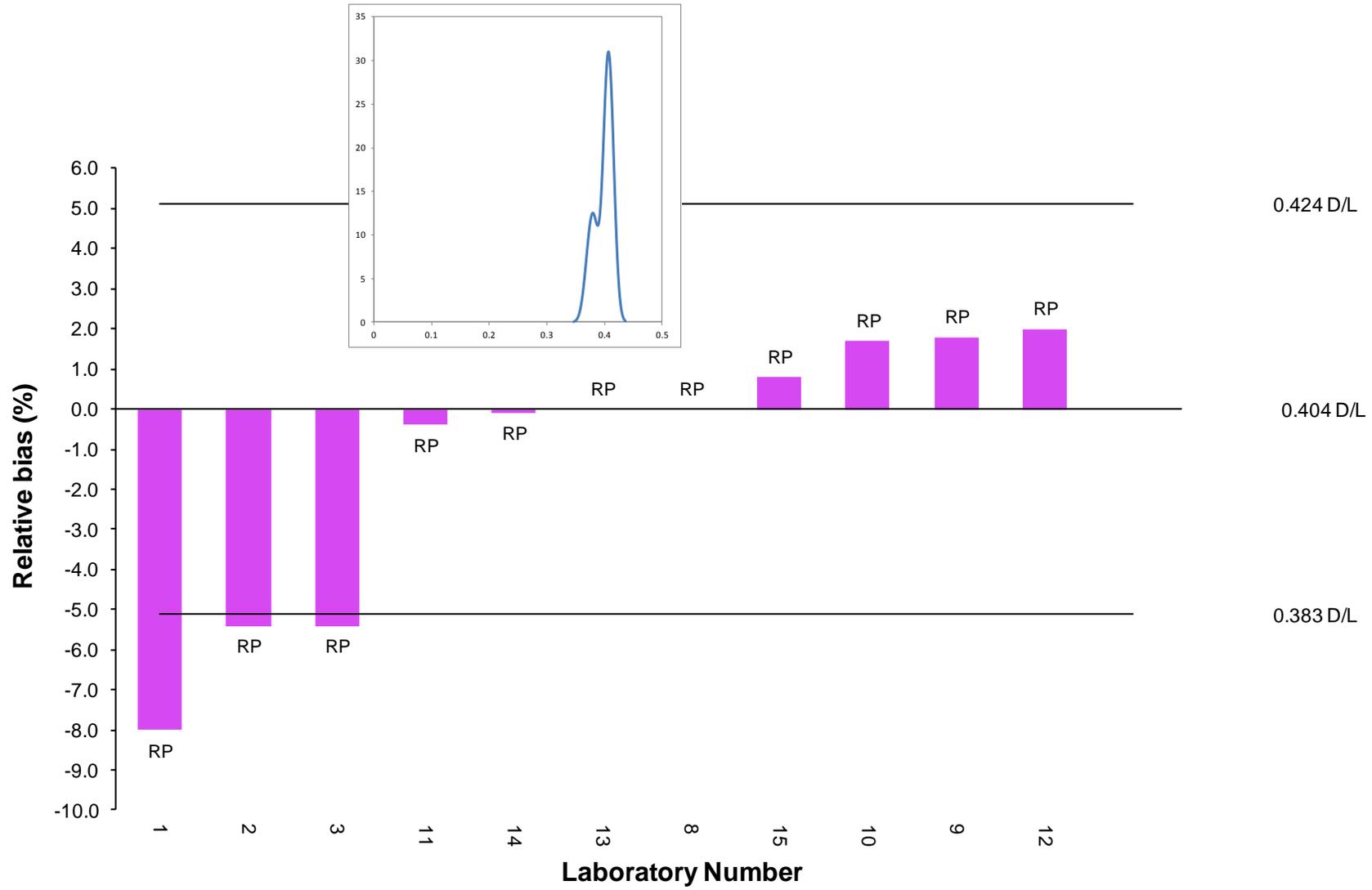


Figure 5.4: Relative Percentage Bias for **Glutamic Acid / Glutamate D/L Results (all data)** in Mollusc Shell (B) Test Material

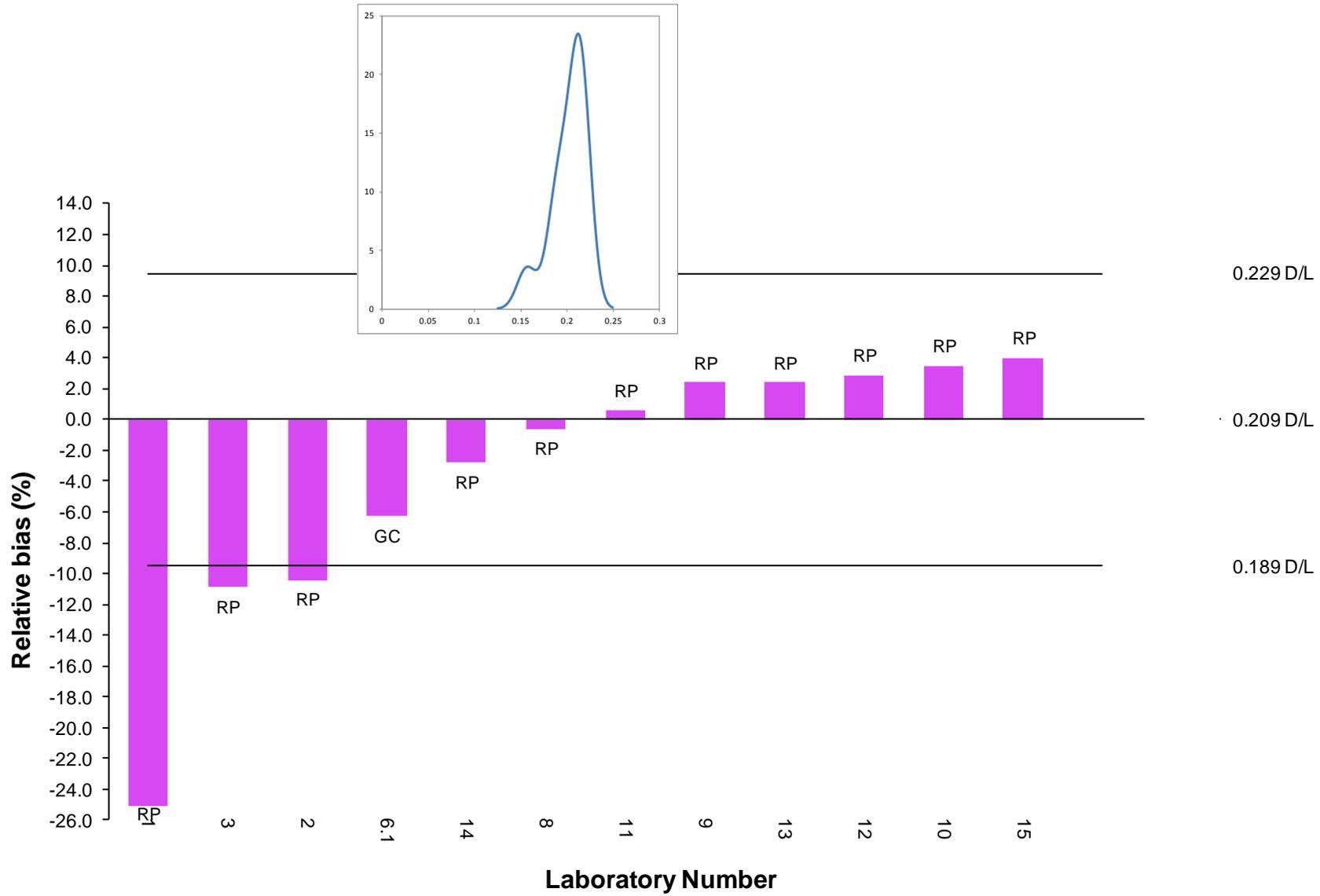


Figure 5.5: Relative Percentage Bias for **Glutamic Acid / Glutamate D/L Results (rpHPLC data only)** in Mollusc Shell (B) Test Material

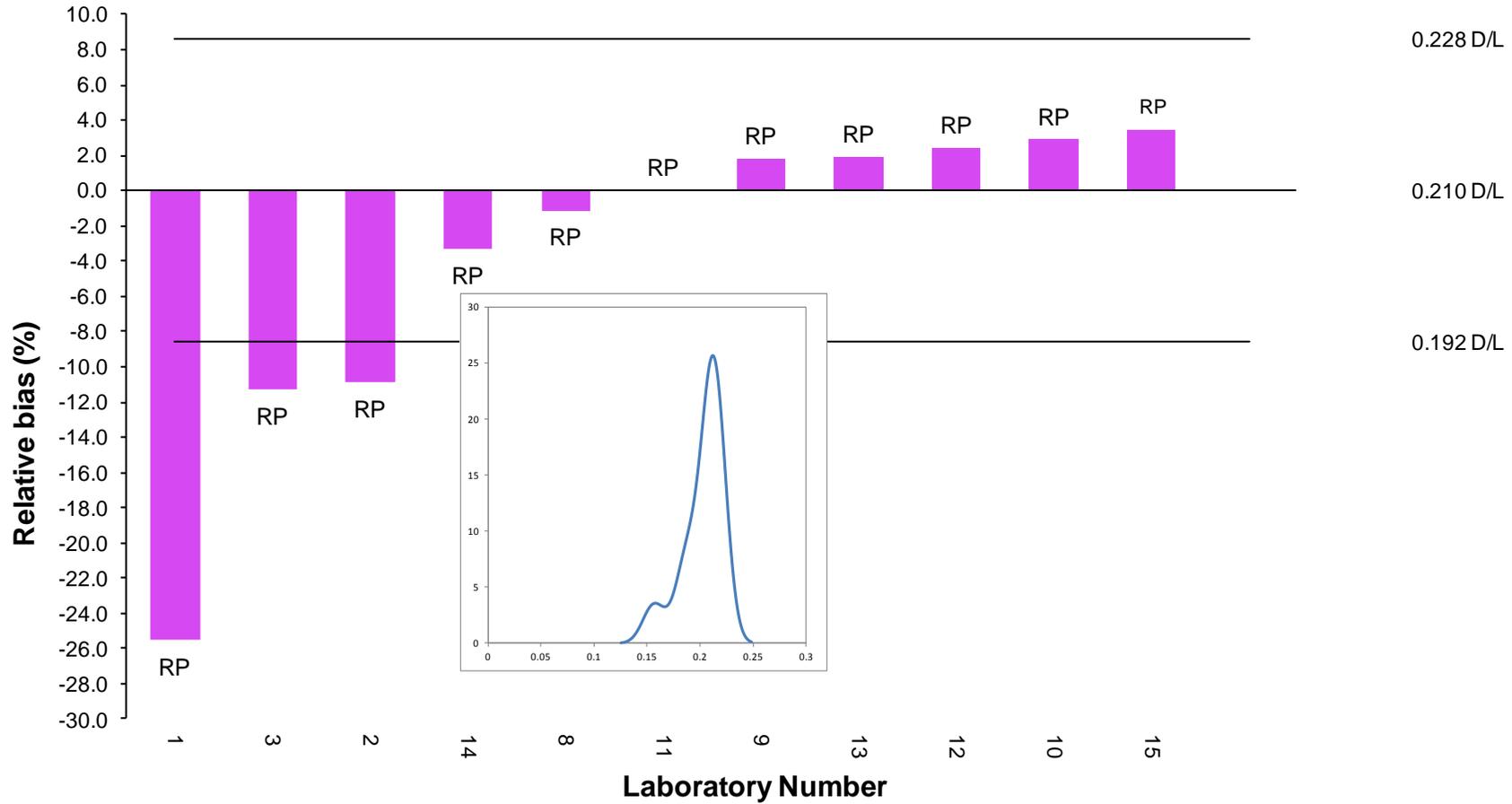


Figure 5.6: Relative Percentage Bias for **Serine D/L Results (all / rpHPLC data)** in Mollusc Shell (B) Test Material

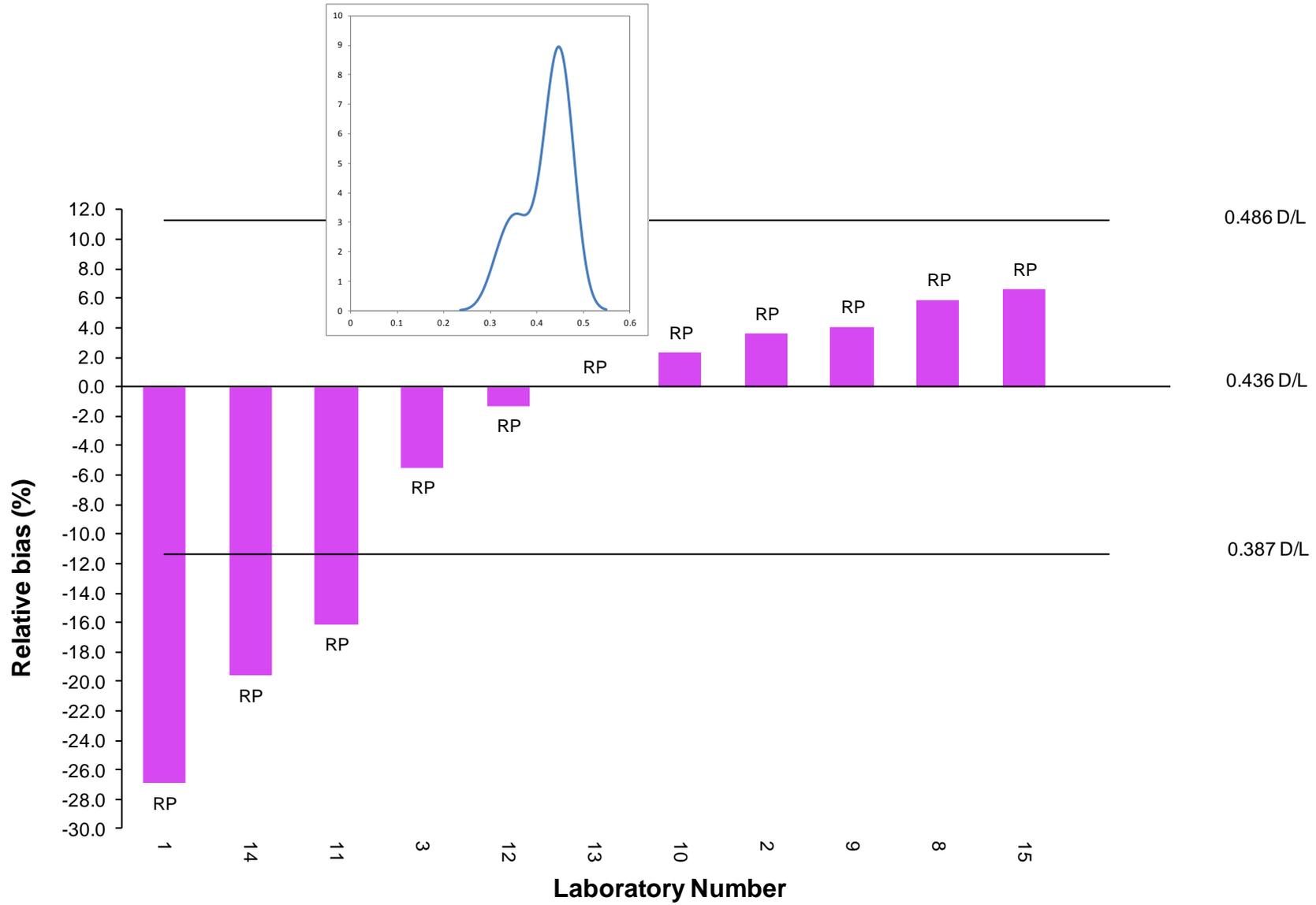


Figure 5.7: Relative Percentage Bias for Arginine D/L Results (rpHPLC data only) in Mollusc Shell (B) Test Material

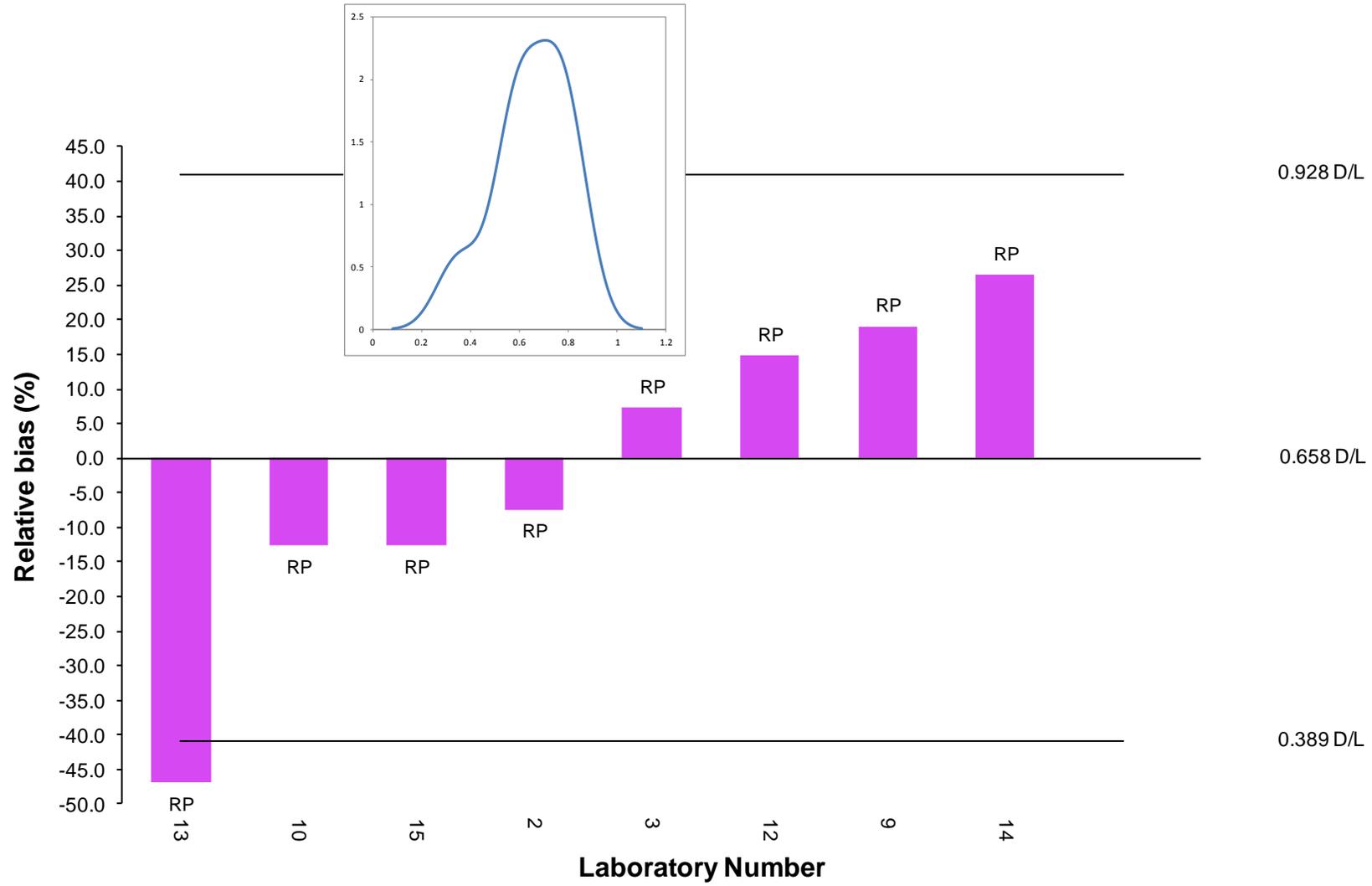


Figure 5.8: Relative Percentage Bias for **Alanine D/L Results (all data)** in Mollusc Shell (B) Test Material

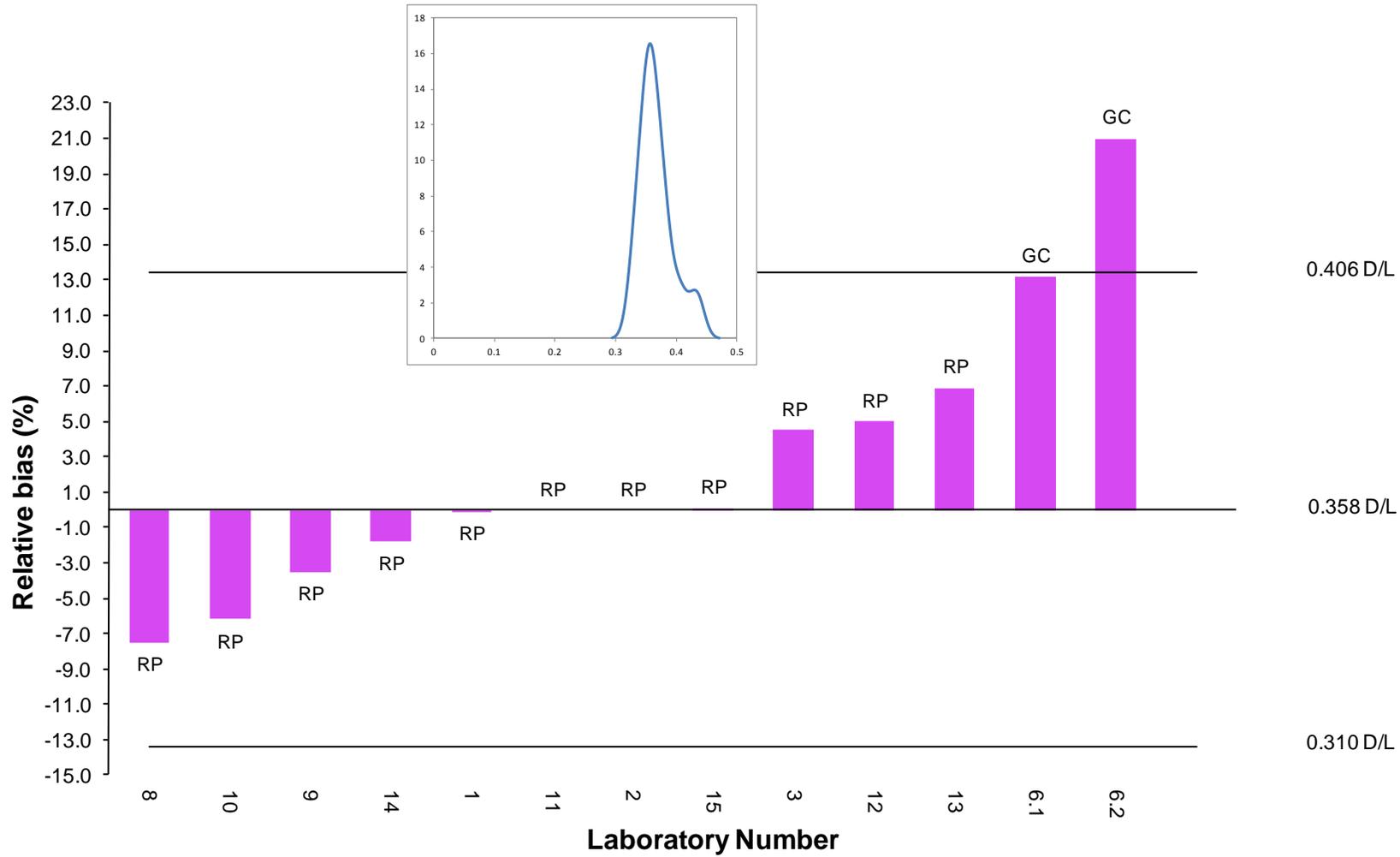


Figure 5.9: Relative Percentage Bias for **Alanine D/L Results (rpHPLC data only)** in Mollusc Shell (B) Test Material

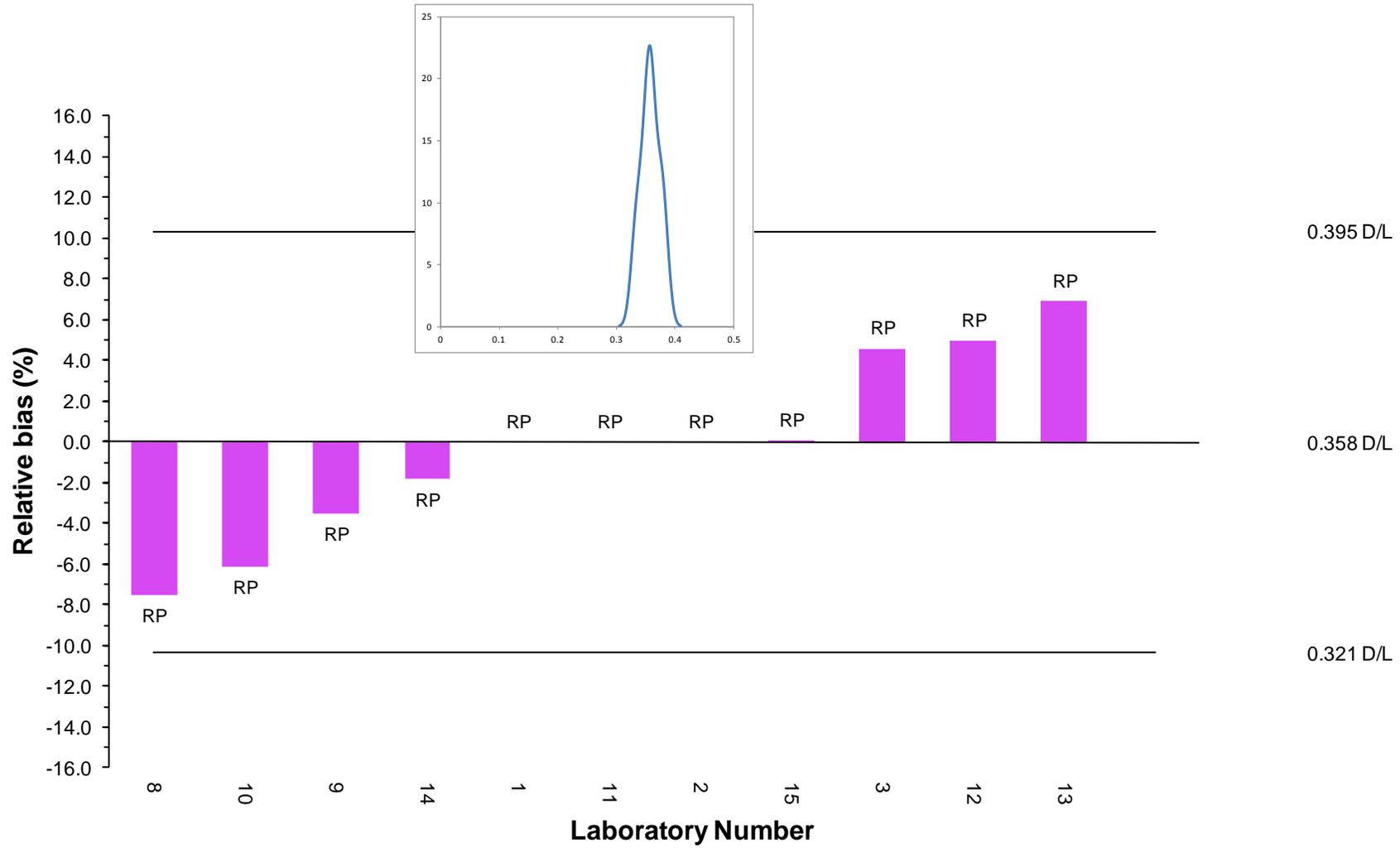


Figure 5.10: Relative Percentage Bias for **Valine D/L Results (all data)** in Mollusc Shell (B) Test Material

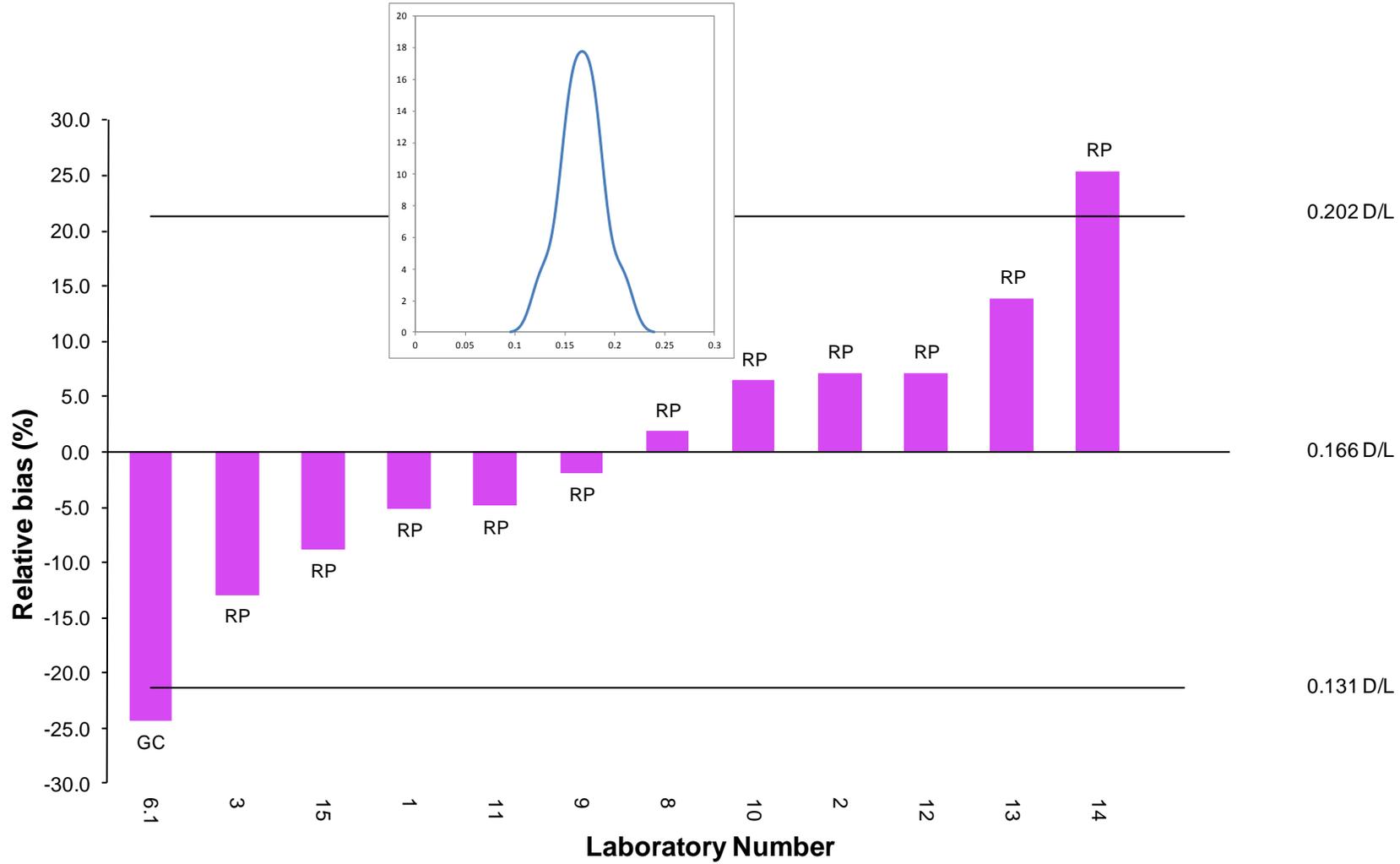


Figure 5.11: Relative Percentage Bias for **Valine D/L Results (rpHPLC data only)** in Mollusc Shell (B) Test Material

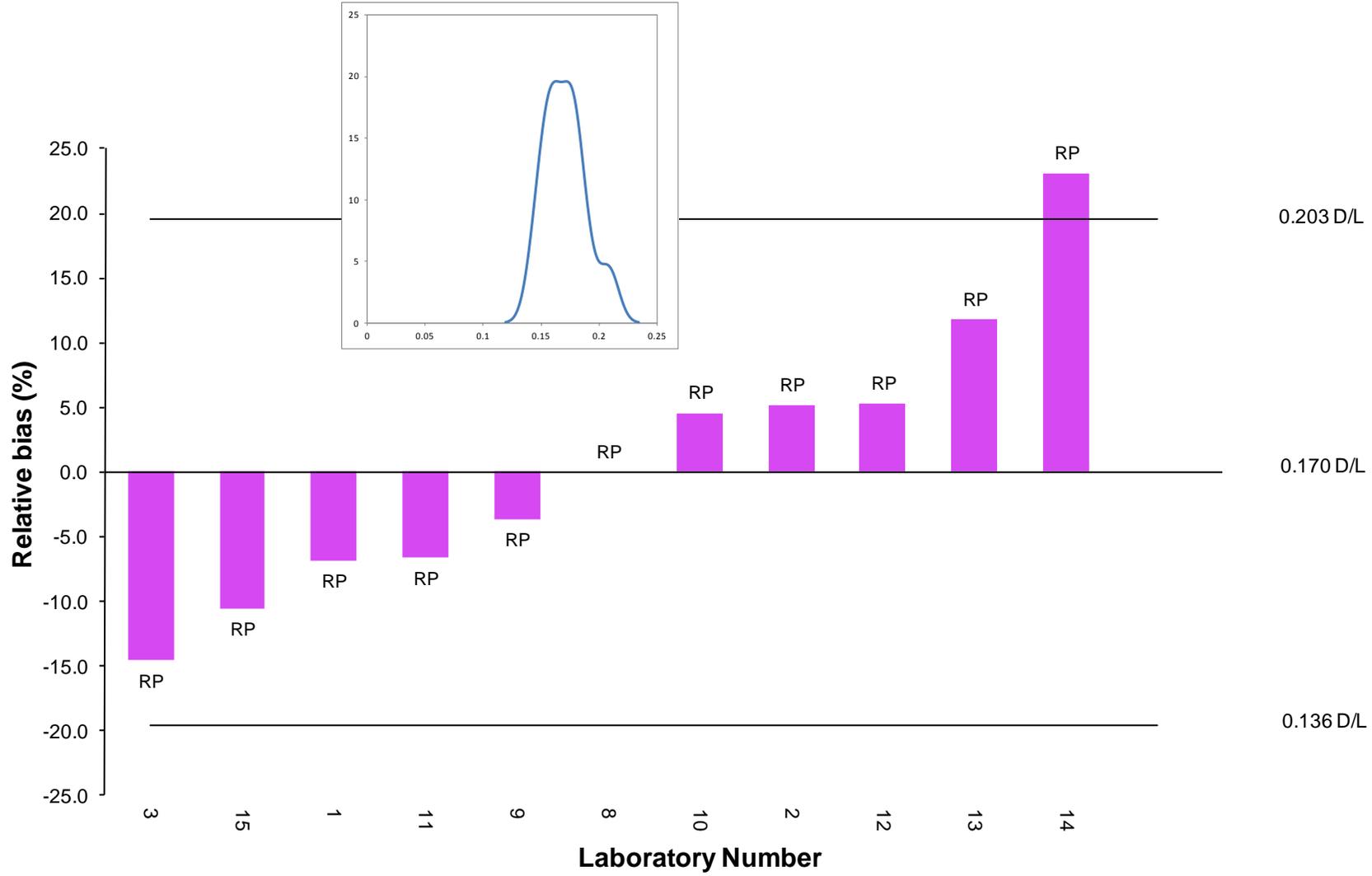


Figure 5.12: Relative Percentage Bias for **Phenylalanine D/L Results (all data)** in Mollusc Shell (B) Test Material

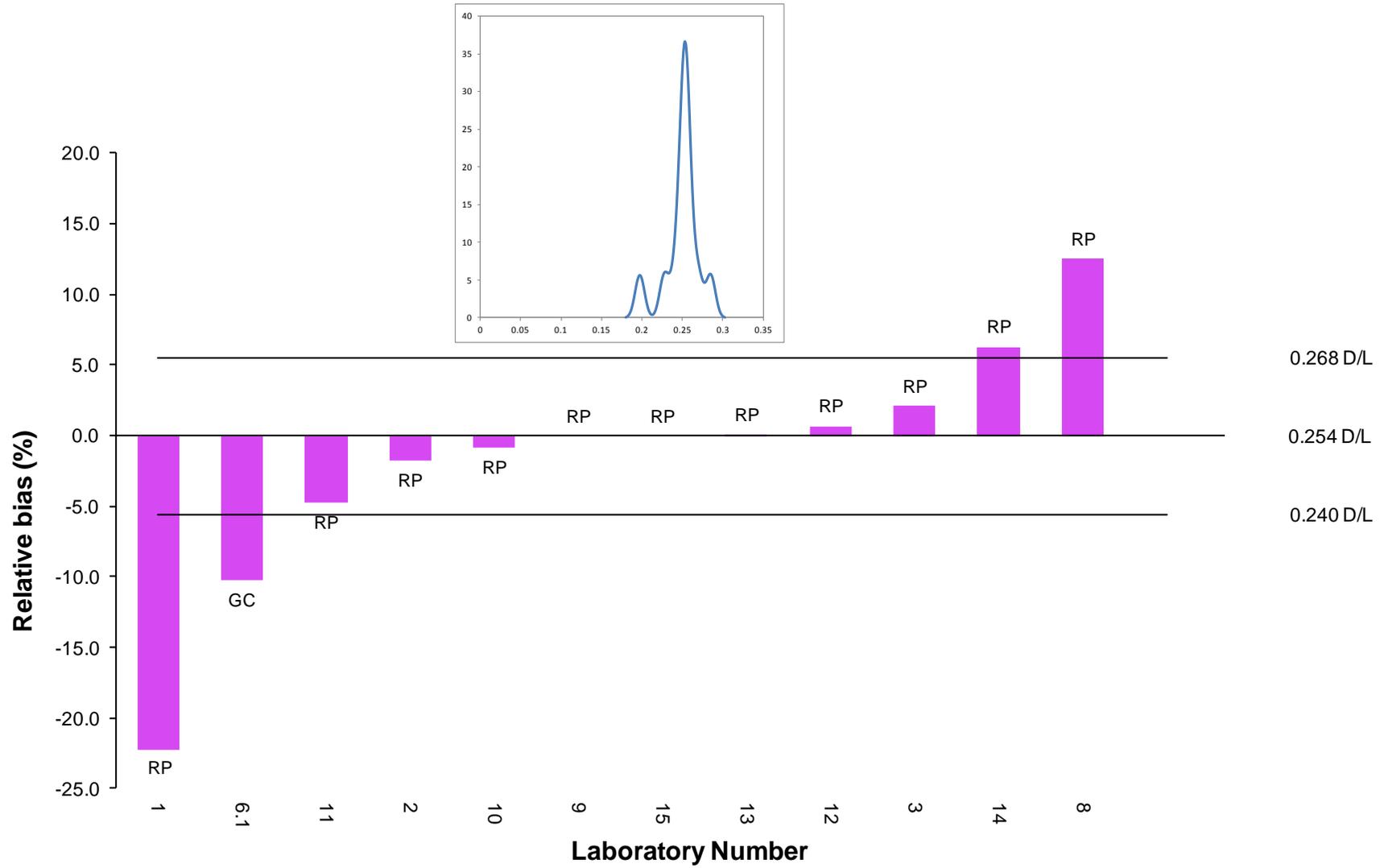


Figure 5.13: Relative Percentage Bias for Phenylalanine D/L Results (rpHPLC data only) in Mollusc Shell (B) Test Material

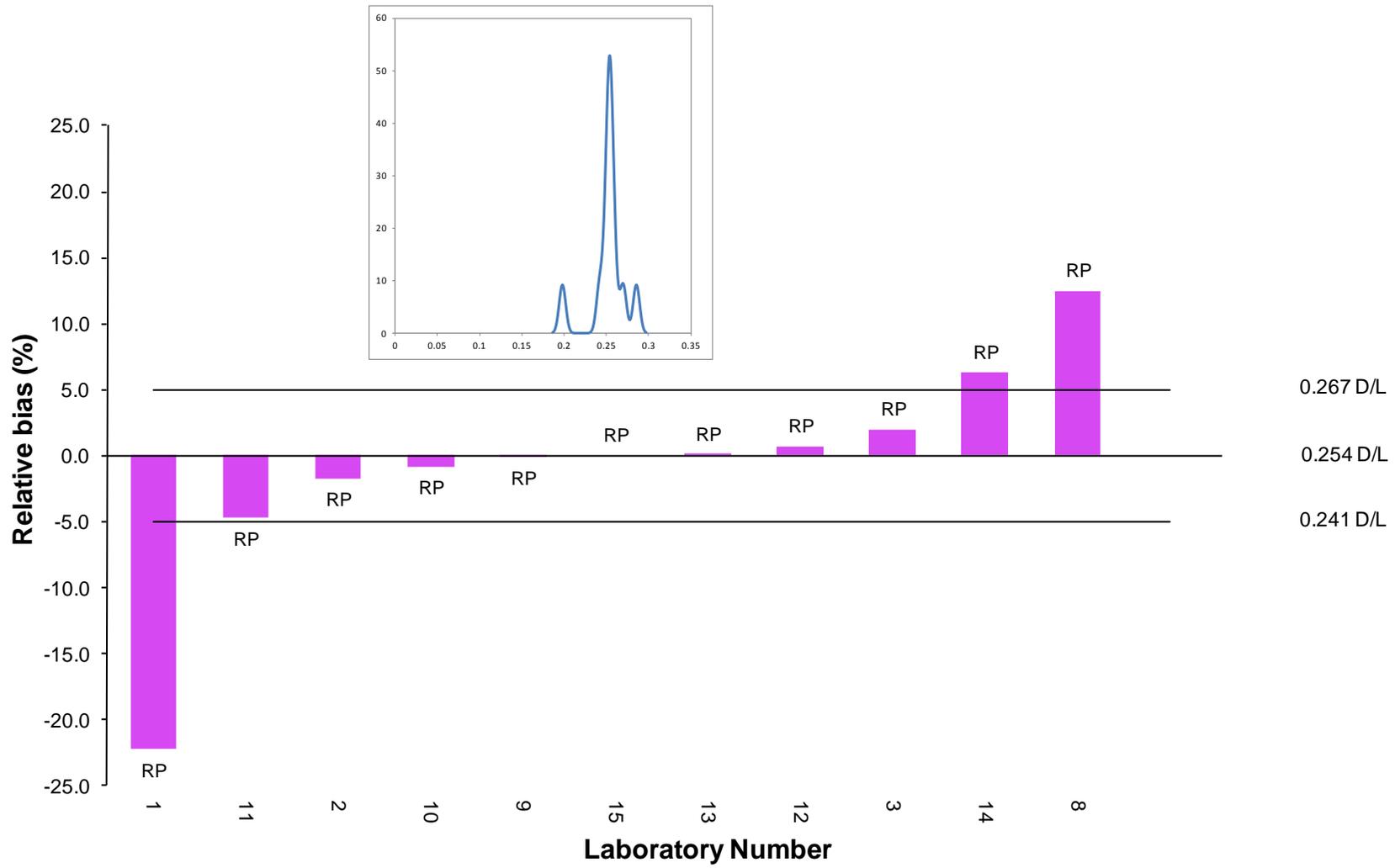


Figure 5.14: Relative Percentage Bias for D-Alloisoleucine/L-Isoleucine Results (all data) in Mollusc Shell (B) Test Material

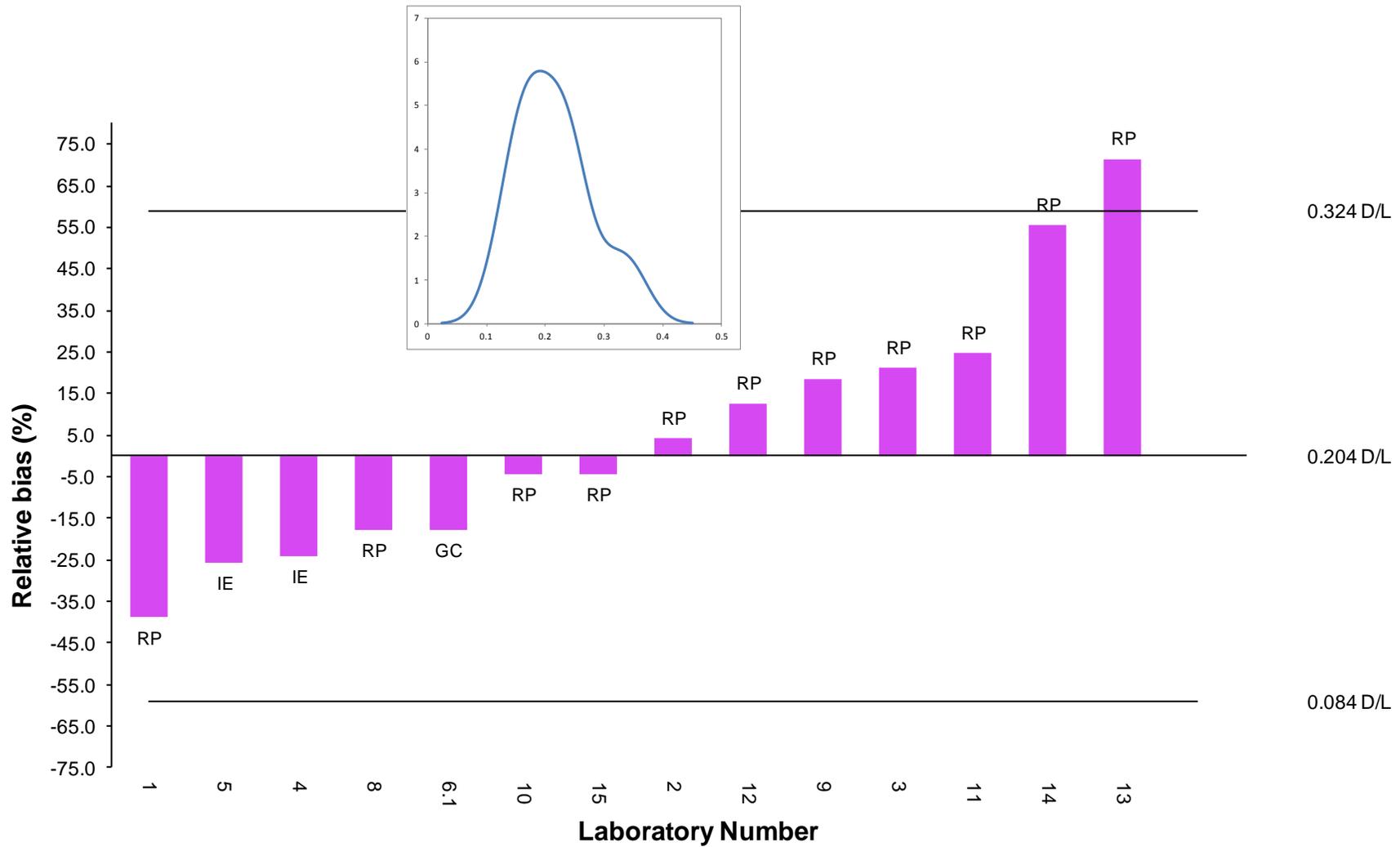


Figure 5.15: Relative Percentage Bias for D-Alloisoleucine/L-Isoleucine Results (rpHPLC data only) in Mollusc Shell (B) Test Material

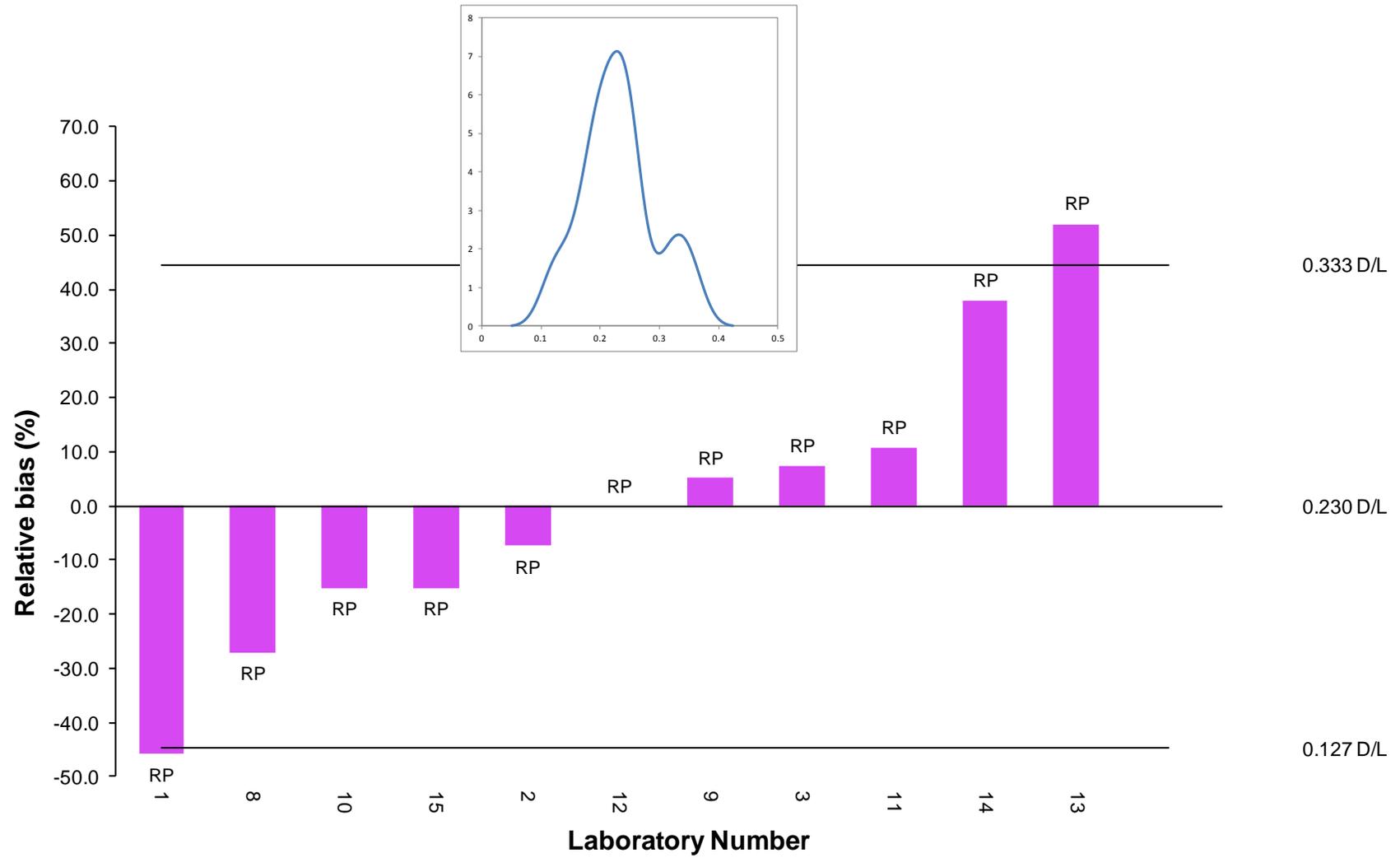


Figure 5.16: Relative Percentage Bias for **Leucine D/L Results (all data)** in Mollusc Shell (B) Test Material

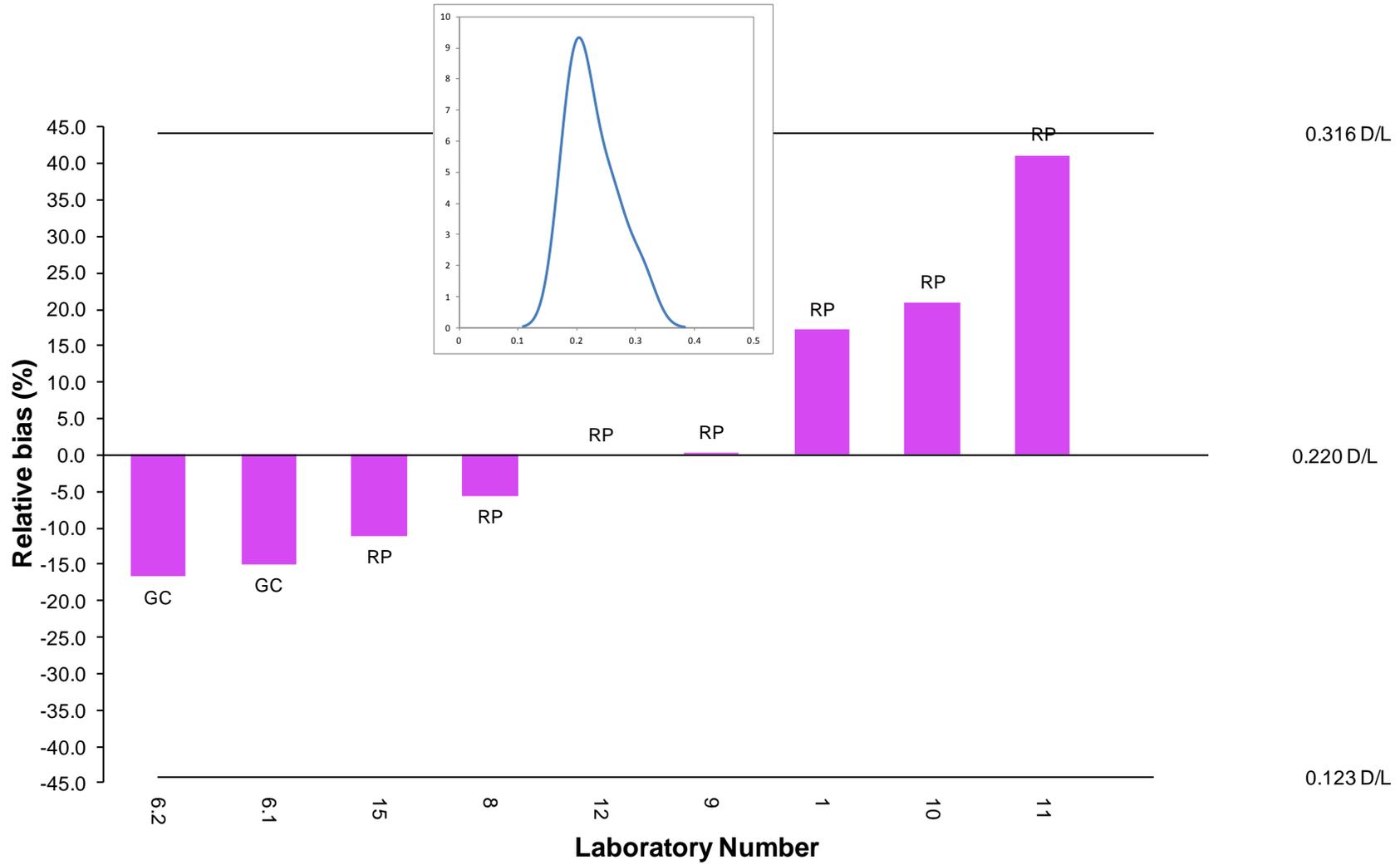


Figure 5.17: Relative Percentage Bias for **Leucine D/L Results (rpHPLC data only)** in Mollusc Shell (B) Test Material

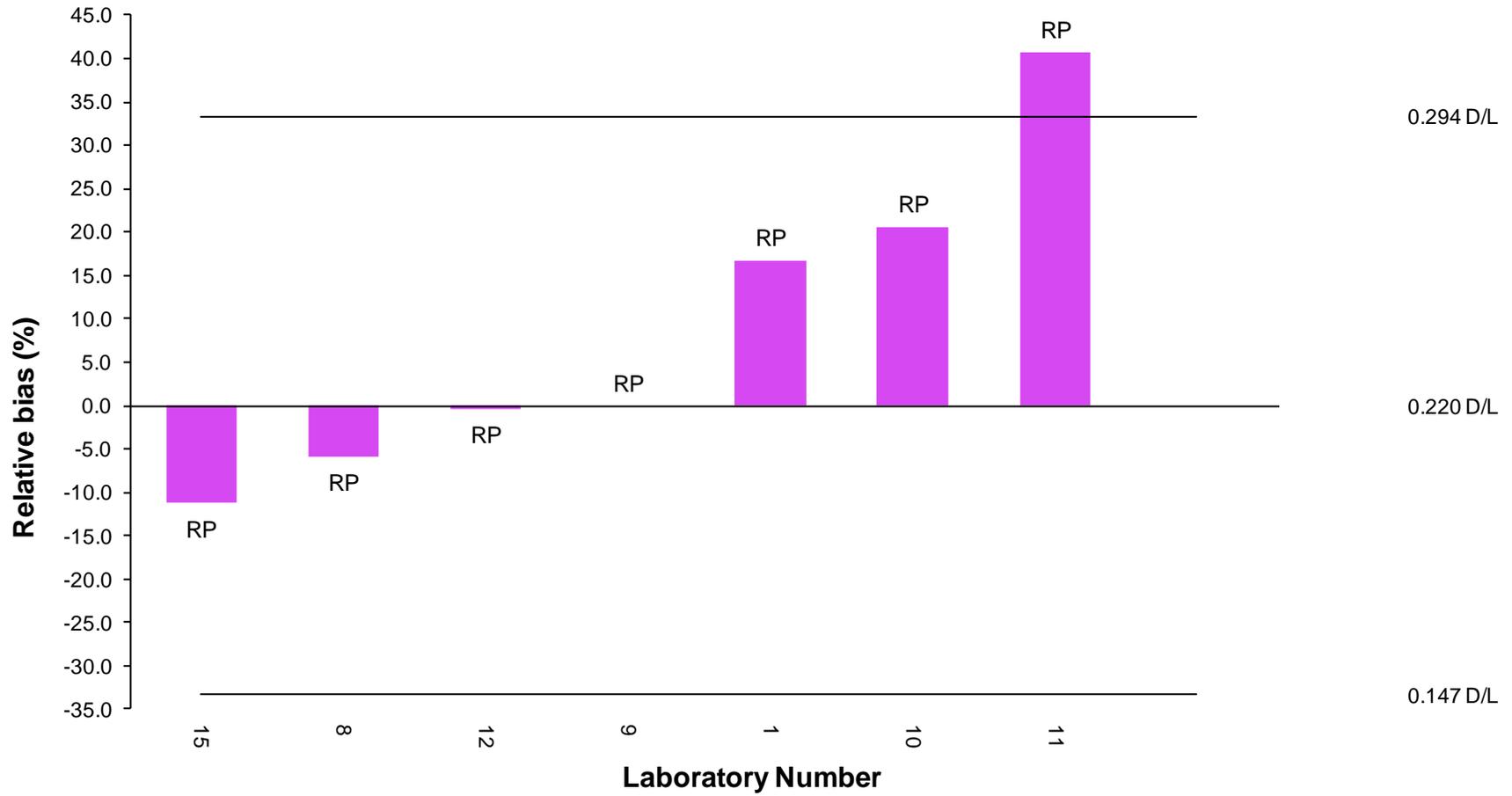
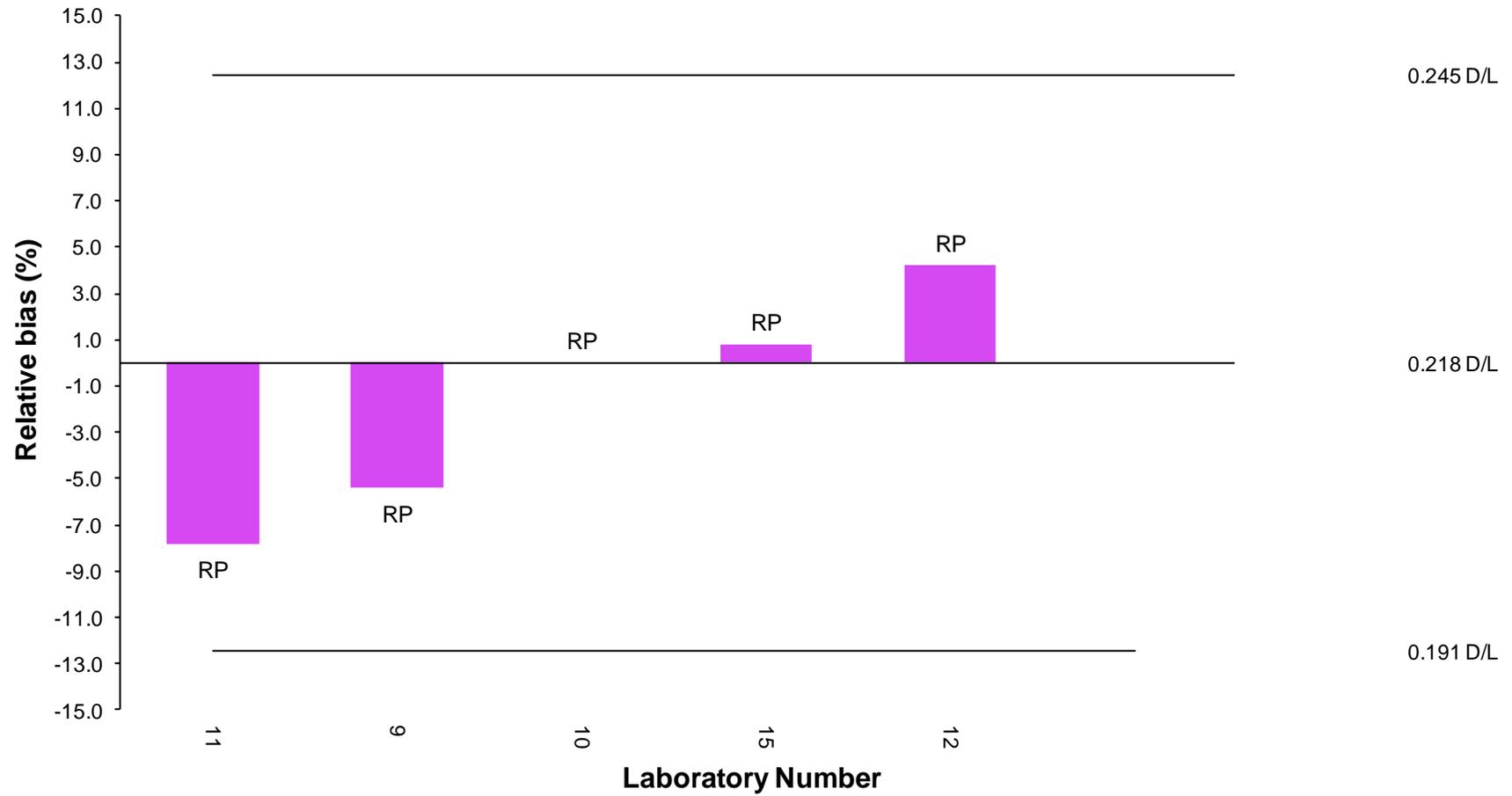


Figure 5.18: Relative Percentage Bias for Tyrosine D/L Results (rpHPLC data only) in Mollusc Shell (B) Test Material



6 MEASUREMENT UNCERTAINTY

Mollusc Shell (B) 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;
$$u_C = \sqrt{S_{RW}^2 + u(bias)^2} \cong S_R$$

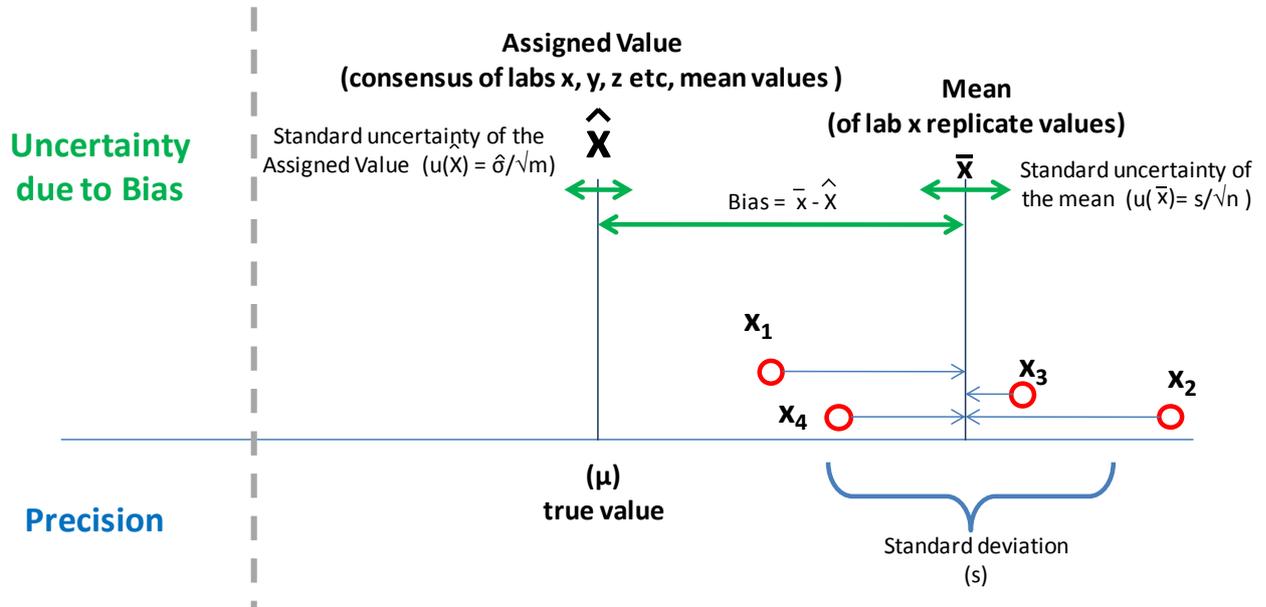
It is widely recognised that evaluation of PT data can be a valuable addition to the determination of measurement uncertainty, however there is very little information provided by the main guidance documents (JCGM 100:, 2008, EURACHEM / CITAC, 2000) on exactly how this should be done. The following methodology is therefore derived from two main sources; the Nordtest Report TR 537ⁱⁱⁱ (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.

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.

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

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 ;

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

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;

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

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;

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

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.; $|z| \leq 2$, 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 contribution S_{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 its 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 .

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

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 Mollusc Shell (B) 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 $(\bar{x}) \pm U$ (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 were 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}		combined $u_c\%$	Expanded $U\% (k = 2)$
	1	2	3		
	$\hat{\sigma}$ as RSD%	$u(\hat{X})$ as RSU%	$RMS_{bias}\%$		
Asx D/L (all ^a)	2.55	0.74	3.39	4.31	8.62
Asx D/L (rpHPLC)	2.52	0.76	3.46	4.35	8.70
Glx D/L (all ^a)	4.75	1.37	8.91	10.19	20.37
Glx D/L (rpHPLC)	4.30	1.30	9.26	10.29	20.58
Ser D/L (rpHPLC)	5.67	1.71	11.73	13.14	26.28
Arg D/L (rpHPLC)	20.49	7.24	22.13	31.02	62.04
Ala D/L (all ^a)	6.69	1.86	7.93	10.54	21.08
Ala D/L (rpHPLC)	5.18	1.56	4.29	6.90	13.80
Val D/L (all ^a)	10.66	3.08	12.49	16.71	33.42
Val D/L (rpHPLC)	9.78	2.95	10.32	14.52	29.04
Phe D/L (all ^a)	2.78	0.80	8.27	8.76	17.52
Phe D/L (rpHPLC)	2.51	0.76	8.08	8.50	16.99
D-Aile/L-Ile (all ^b)	29.43	7.86	30.66	43.22	86.43
D-Aile/L-Ile (rpHPLC)	22.32	6.73	26.43	35.24	70.49
Leu D/L (all ^a)	22.04	7.35	18.51	29.71	59.41
Leu D/L (rpHPLC)	16.66	6.30	19.01	26.05	52.10
Tyr D/L (rpHPLC)	6.22	2.78	4.65	8.25	16.50

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				Combined & Expanded uncertainties	
		Precision ⁴	Bias components ^{5,6,7}			combined $u_c\%$	Expanded $U\% (k = 2)$
Asx D/L	σ std dev as CV% ⁴	$u(\hat{X})$ as RSU% ⁵	$u(\bar{x})$ as RSU% ⁶	Relative bias % ⁷			
1	0.372	0.21	0.74	0.07	7.98	8.02	16.04
2	0.382	1.18	0.74	0.68	5.40	5.62	11.24
3	0.382	n=1	0.74	n=1	5.40		
4							
5							
6.1	0.413	5.81	0.74	2.05	2.27	6.61	13.22
6.2							
8	0.404	0.70	0.74	0.50	0.04	1.13	2.26
9	0.411	0.21	0.74	0.15	1.79	1.95	3.90
10	0.411	0.60	0.74	0.42	1.66	1.96	3.91
11	0.402	0.00	0.74	0.00	0.44	0.86	1.72
12	0.412	0.18	0.74	0.13	2.04	2.18	4.36
13	0.404	n=1	0.74	n=1	0.04		
14	0.403	n=1	0.74	n=1	0.08		
15	0.407	0.69	0.74	0.48	0.76	1.35	2.70
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	0.372	0.21	0.76	0.07	7.94	7.98	15.97
2	0.382	1.18	0.76	0.68	5.36	5.58	11.17
3	0.382	n=1	0.76	n=1	5.36		
4							
5							
6.1							
6.2							
8	0.404	0.70	0.76	0.50	0.09	1.15	2.30
9	0.411	0.21	0.76	0.15	1.83	2.00	4.00
10	0.411	0.60	0.76	0.42	1.70	2.00	4.01
11	0.402	0.00	0.76	0.00	0.40	0.86	1.72
12	0.412	0.18	0.76	0.13	2.09	2.23	4.46
13	0.404	n=1	0.76	n=1	0.00		
14	0.403	n=1	0.76	n=1	0.04		
15	0.407	0.69	0.76	0.48	0.80	1.39	2.78

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				Combined & Expanded uncertainties	
		Precision ⁴		Bias components ^{5,6,7}		combined $u_c\%$	Expanded $U\%$ ($k = 2$)
Glx D/L	σ std dev as CV% ⁴	$u(\hat{X})$ as RSU% ⁵	$u(\bar{x})$ as RSU% ⁶	Relative bias % ⁷			
1	0.157	0.84	1.37	0.27	25.10	25.16	50.31
2	0.187	0.69	1.37	0.40	10.38	10.50	21.00
3	0.186	n=1	1.37	n=1	10.85		
4							
5							
6.1	0.196	2.04	1.37	0.72	6.29	6.79	13.59
6.2							
8	0.208	3.40	1.37	2.40	0.56	4.42	8.84
9	0.214	0.22	1.37	0.16	2.39	2.77	5.53
10	0.216	0.06	1.37	0.04	3.47	3.73	7.46
11	0.210	0.69	1.37	0.49	0.56	1.70	3.41
12	0.215	0.11	1.37	0.08	2.93	3.24	6.48
13	0.214	n=1	1.37	n=1	2.42		
14	0.203	n=1	1.37	n=1	2.78		
15	0.218	0.02	1.37	0.01	4.03	4.25	8.50
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	0.157	0.84	1.30	0.27	25.52	25.57	51.13
2	0.187	0.69	1.30	0.40	10.87	10.98	21.96
3	0.186	n=1	1.30	n=1	11.35		
4							
5							
6.1							
6.2							
8	0.208	3.40	1.30	2.40	1.11	4.50	9.00
9	0.214	0.22	1.30	0.16	1.82	2.25	4.50
10	0.216	0.06	1.30	0.04	2.90	3.17	6.35
11	0.210	0.69	1.30	0.49	0.00	1.55	3.10
12	0.215	0.11	1.30	0.08	2.36	2.70	5.40
13	0.214	n=1	1.30	n=1	1.86		
14	0.203	n=1	1.30	n=1	3.32		
15	0.218	0.02	1.30	0.01	3.45	3.69	7.37

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				Combined & Expanded uncertainties	
		Precision ⁴		Bias components ^{5,6,7}		combined $u_c\%$	Expanded $U\%$ ($k = 2$)
Ser D/L	σ std dev as CV% ⁴	$u(\hat{X})$ as RSU% ⁵	$u(\bar{x})$ as RSU% ⁶	Relative bias % ⁷			
1	0.319	0.90	1.71	0.28	26.88	26.95	53.89
2	0.452	1.21	1.71	0.70	3.57	4.20	8.40
3	0.412	n=1	1.71	n=1	5.54		
4							
5							
6.1							
6.2							
8	0.462	7.04	1.71	4.98	5.87	10.57	21.14
9	0.454	1.24	1.71	0.88	4.13	4.72	9.43
10	0.447	2.33	1.71	1.65	2.35	4.07	8.14
11	0.366	0.80	1.71	0.57	16.13	16.25	32.50
12	0.431	0.72	1.71	0.51	1.32	2.33	4.66
13	0.436	n=1	1.71	n=1	0.00		
14	0.351	n=1	1.71	n=1	19.64		
15	0.465	1.73	1.71	1.22	6.61	7.15	14.29
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	0.610	0.50	7.24	0.29	7.38	10.36	20.71
3	0.707	n=1	7.24	n=1	7.38		
4							
5							
6.1							
6.2							
8							
9	0.784	33.96	7.24	24.02	19.07	46.33	92.66
10	0.575	25.53	7.24	18.05	12.70	34.52	69.03
11							
12	0.757	26.23	7.24	18.55	14.94	36.16	72.33
13	0.350	n=1	7.24	n=1	46.89		
14	0.833	n=1	7.24	n=1	26.47		
15	0.575	21.60	7.24	15.28	12.69	30.22	60.45

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				Combined & Expanded uncertainties	
		Precision ⁴		Bias components ^{5,6,7}		combined $u_c\%$	Expanded $U\%$ ($k = 2$)
Ala D/L		σ std dev as CV% ⁴	$u(\hat{X})$ as RSU% ⁵	$u(\bar{x})$ as RSU% ⁶	Relative bias % ⁷		
1	0.358	2.63	1.86	0.83	0.08	3.32	6.65
2	0.358	1.02	1.86	0.59	0.00	2.20	4.39
3	0.374	n=1	1.86	n=1	4.51		
4							
5							
6.1	0.405	6.42	1.86	3.21	13.18	15.12	30.24
6.2	0.433	7.39	1.86	3.02	21.00	22.55	45.09
8	0.331	4.27	1.86	3.02	7.50	9.33	18.66
9	0.345	0.82	1.86	0.58	3.53	4.11	8.23
10	0.336	1.71	1.86	1.21	6.18	6.79	13.58
11	0.358	0.65	1.86	0.46	0.04	2.02	4.04
12	0.376	3.13	1.86	2.21	4.98	6.55	13.11
13	0.382	n=1	1.86	n=1	6.88		
14	0.351	n=1	1.86	n=1	1.84		
15	0.358	0.95	1.86	0.67	0.08	2.19	4.39
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	0.358	2.63	1.56	0.83	0.04	3.17	6.34
2	0.358	1.02	1.56	0.59	0.04	1.95	3.91
3	0.374	n=1	1.56	n=1	4.56		
4							
5							
6.1							
6.2							
8	0.331	4.27	1.56	3.02	7.46	9.25	18.49
9	0.345	0.82	1.56	0.58	3.49	3.95	7.91
10	0.336	1.71	1.56	1.21	6.14	6.68	13.36
11	0.358	0.65	1.56	0.46	0.00	1.75	3.50
12	0.376	3.13	1.56	2.21	5.03	6.51	13.02
13	0.382	n=1	1.56	n=1	6.92		
14	0.351	n=1	1.56	n=1	1.80		
15	0.358	0.95	1.56	0.67	0.12	1.95	3.90

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				Combined & Expanded uncertainties	
		Precision ⁴		Bias components ^{5,6,7}		combined $u_c\%$	Expanded $U\%$ ($k = 2$)
Val D/L		σ std dev as CV% ⁴	$u(\hat{X})$ as RSU% ⁵	$u(\bar{x})$ as RSU% ⁶	Relative bias % ⁷		
1	0.158	7.71	3.08	2.44	5.06	10.03	20.06
2	0.178	0.47	3.08	0.27	7.18	7.83	15.65
3	0.145	n=1	3.08	n=1	12.90		
4							
5							
6.1	0.126	3.17	3.08	0.92	24.29	24.70	49.41
6.2							
8	0.170	3.75	3.08	2.65	1.85	5.83	11.67
9	0.163	3.08	3.08	2.18	1.85	5.21	10.42
10	0.177	3.19	3.08	2.25	6.50	8.18	16.37
11	0.158	0.20	3.08	0.14	4.87	5.77	11.53
12	0.178	4.12	3.08	2.91	7.21	9.32	18.65
13	0.189	n=1	3.08	n=1	13.85		
14	0.209	n=1	3.08	n=1	25.37		
15	0.152	0.94	3.08	0.67	8.85	9.44	18.88
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	0.158	7.71	2.95	2.44	6.79	10.97	21.93
2	0.178	0.47	2.95	0.27	5.23	6.03	12.05
3	0.145	n=1	2.95	n=1	14.48		
4							
5							
6.1							
6.2							
8	0.170	3.75	2.95	2.65	0.00	5.46	10.93
9	0.163	3.08	2.95	2.18	3.63	6.01	12.02
10	0.177	3.19	2.95	2.25	4.57	6.69	13.38
11	0.158	0.20	2.95	0.14	6.60	7.23	14.46
12	0.178	4.12	2.95	2.91	5.26	7.86	15.73
13	0.189	n=1	2.95	n=1	11.79		
14	0.209	n=1	2.95	n=1	23.09		
15	0.152	0.94	2.95	0.67	10.50	10.97	21.94

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				Combined & Expanded uncertainties	
		Precision ⁴		Bias components ^{5,6,7}		combined $u_c\%$	Expanded $U\%$ ($k = 2$)
Phe D/L		σ std dev as CV% ⁴	$u(\hat{X})$ as RSU% ⁵	$u(\bar{x})$ as RSU% ⁶	Relative bias % ⁷		
1	0.198	13.18	0.80	4.17	22.16	26.13	52.26
2	0.250	3.42	0.80	1.97	1.65	4.35	8.70
3	0.259	n=1	0.80	n=1	2.09		
4							
5							
6.1	0.228	4.82	0.80	1.29	10.16	11.35	22.69
6.2							
8	0.286	4.21	0.80	2.98	12.50	13.55	27.09
9	0.254	0.89	0.80	0.63	0.04	1.35	2.70
10	0.252	0.72	0.80	0.51	0.78	1.43	2.85
11	0.242	1.58	0.80	1.12	4.68	5.13	10.25
12	0.256	0.59	0.80	0.42	0.73	1.30	2.61
13	0.254	n=1	0.80	n=1	0.10		
14	0.270	n=1	0.80	n=1	6.30		
15	0.254	3.57	0.80	2.53	0.04	4.45	8.90
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	0.198	13.18	0.76	4.17	22.20	26.16	52.32
2	0.250	3.42	0.76	1.97	1.69	4.36	8.72
3	0.259	n=1	0.76	n=1	2.05		
4							
5							
6.1							
6.2							
8	0.286	4.21	0.76	2.98	12.45	13.50	27.00
9	0.254	0.89	0.76	0.63	0.09	1.33	2.66
10	0.252	0.72	0.76	0.51	0.83	1.43	2.86
11	0.242	1.58	0.76	1.12	4.72	5.16	10.31
12	0.256	0.59	0.76	0.42	0.69	1.25	2.51
13	0.254	n=1	0.76	n=1	0.06		
14	0.270	n=1	0.76	n=1	6.25		
15	0.254	3.57	0.76	2.53	0.00	4.44	8.88

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				Combined & Expanded uncertainties	
		Precision ⁴		Bias components ^{5,6,7}		combined $u_c\%$	Expanded $U\%$ ($k = 2$)
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			
1	0.125	4.51	7.86	1.43	38.88	39.94	79.89
2	0.213	2.60	7.86	1.50	4.31	9.46	18.92
3	0.247	n=1	7.86	n=1	21.14		
4	0.155	5.47	7.86	3.87	24.11	26.24	52.47
5	0.152	0.00	7.86	0.00	25.58	26.76	53.53
6.1	0.168	3.57	7.86	0.95	17.75	19.76	39.53
6.2							
8	0.168	5.89	7.86	4.17	17.75	20.71	41.42
9	0.242	23.70	7.86	16.76	18.55	35.33	70.67
10	0.195	6.97	7.86	4.93	4.35	12.40	24.80
11	0.255	8.43	7.86	5.96	24.79	27.98	55.96
12	0.230	18.64	7.86	13.18	12.65	27.26	54.52
13	0.350	n=1	7.86	n=1	71.35		
14	0.317	n=1	7.86	n=1	55.40		
15	0.195	14.68	7.86	10.38	4.31	20.09	40.18
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	0.125	4.51	6.73	1.43	45.74	46.47	92.95
2	0.213	2.60	6.73	1.50	7.40	10.45	20.90
3	0.247	n=1	6.73	n=1	7.54		
4							
5							
6.1							
6.2							
8	0.168	5.89	6.73	4.17	26.99	28.73	57.47
9	0.242	23.70	6.73	16.76	5.24	30.25	60.51
10	0.195	6.97	6.73	4.93	15.09	18.60	37.20
11	0.255	8.43	6.73	5.96	10.77	16.37	32.74
12	0.230	18.64	6.73	13.18	0.00	23.80	47.60
13	0.350	n=1	6.73	n=1	52.11		
14	0.317	n=1	6.73	n=1	37.95		
15	0.195	14.68	6.73	10.38	15.06	24.39	48.79

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				Combined & Expanded uncertainties	
		Precision ⁴		Bias components ^{5,6,7}		combined $u_c\%$	Expanded $U\%$ ($k = 2$)
Leu D/L		σ std dev as CV% ⁴	$u(\hat{X})$ as RSU% ⁵	$u(\bar{x})$ as RSU% ⁶	Relative bias % ⁷		
1	0.257	12.65	7.35	4.00	17.21	22.94	45.88
2							
3							
4							
5							
6.1	0.187	6.42	7.35	1.72	14.87	17.86	35.73
6.2	0.183	3.28	7.35	1.64	16.69	18.60	37.20
8	0.208	6.47	7.35	4.58	5.53	12.15	24.29
9	0.220	1.91	7.35	1.35	0.31	7.72	15.43
10	0.266	26.87	7.35	19.00	21.02	39.74	79.47
11	0.310	3.80	7.35	2.68	41.19	42.10	84.19
12	0.220	2.57	7.35	1.82	0.00	7.99	15.99
13							
14							
15	0.196	7.00	7.35	4.95	10.96	15.74	31.47
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	0.257	12.65	6.30	4.00	16.85	22.35	44.70
2							
3							
4							
5							
6.1							
6.2							
8	0.208	6.47	6.30	4.58	5.83	11.68	23.37
9	0.220	1.91	6.30	1.35	0.00	6.72	13.43
10	0.266	26.87	6.30	19.00	20.64	39.36	78.71
11	0.310	3.80	6.30	2.68	40.75	41.49	82.99
12	0.220	2.57	6.30	1.82	0.31	7.05	14.10
13							
14							
15	0.196	7.00	6.30	4.95	11.24	15.47	30.95

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				Combined & Expanded uncertainties	
		Precision ⁴		Bias components ^{5,6,7}		combined $u_c\%$	Expanded $U\%$ ($k = 2$)
Try D/L	σ std dev as CV% ⁴	$u(\hat{X})$ as RSU% ⁵	$u(\bar{x})$ as RSU% ⁶	Relative bias % ⁷			
1							
2							
3							
4							
5							
6.1							
6.2							
8							
9	0.206	2.85	2.78	2.02	5.41	7.01	14.03
10	0.218	1.65	2.78	1.17	0.00	3.44	6.88
11	0.201	n=1	2.78	n=1	7.78		
12	0.227	1.67	2.78	1.18	4.19	5.43	10.86
13							
14							
15	0.220	1.13	2.78	0.80	0.82	3.22	6.43

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.; $Bias\% = (\bar{x} - \hat{X}/\hat{X}) \times 100$

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 Mollusc Shell (B) Test Material

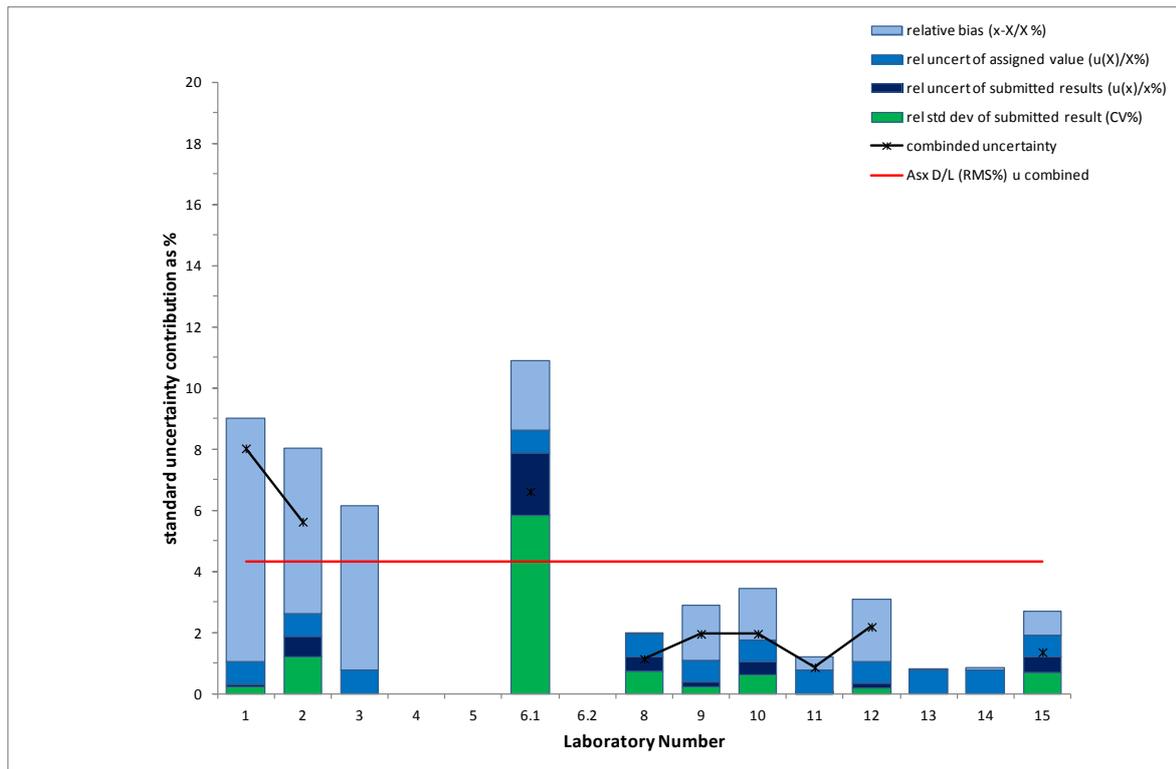


Figure 6.3: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Aspartic acid / Asparagine** D/L Values in Mollusc Shell (B) Test Material

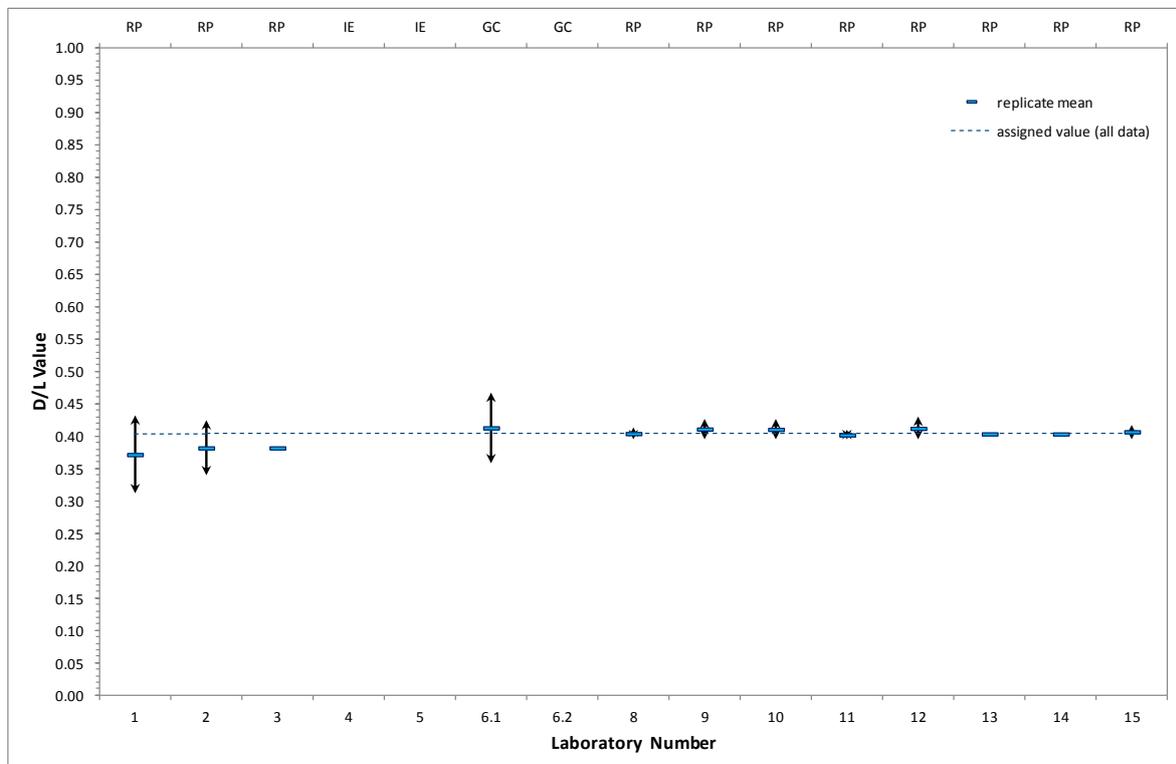


Figure 6.4: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Aspartic acid / Asparagine** rpHPLC D/L Values in Mollusc Shell (B) Test Material

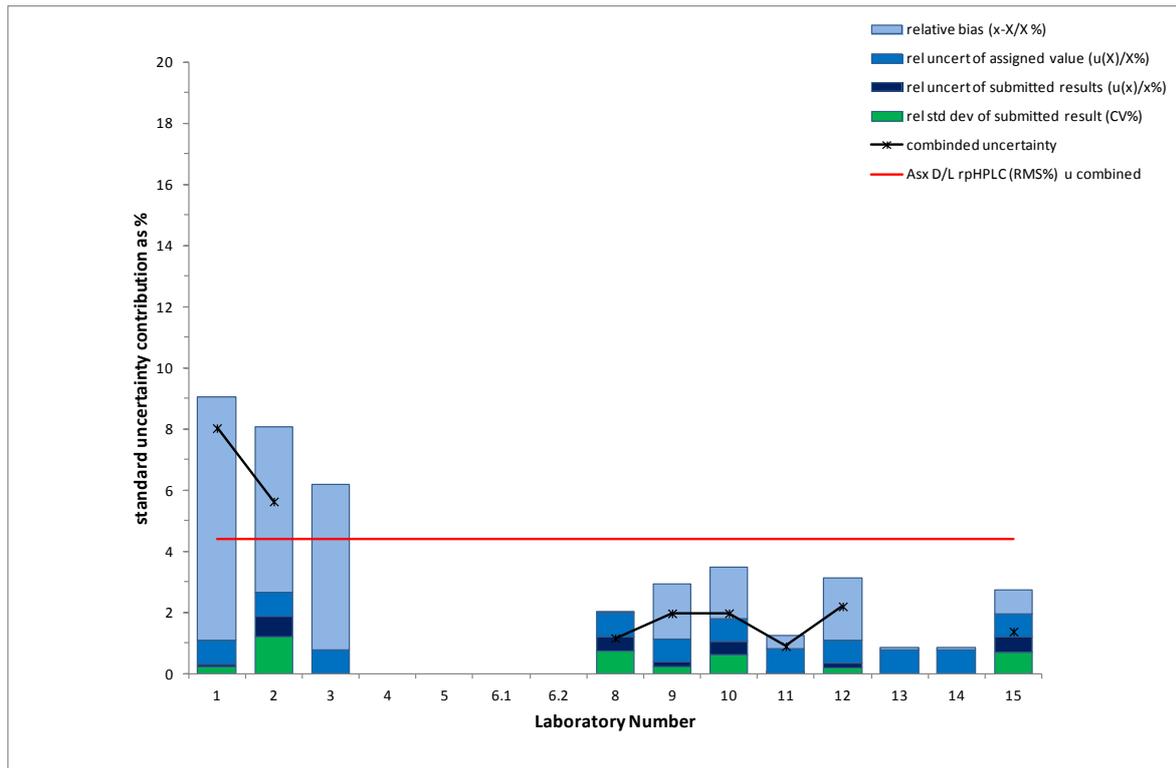


Figure 6.5: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Aspartic acid / Asparagine** rpHPLC D/L Values in Mollusc Shell (B) Test Material

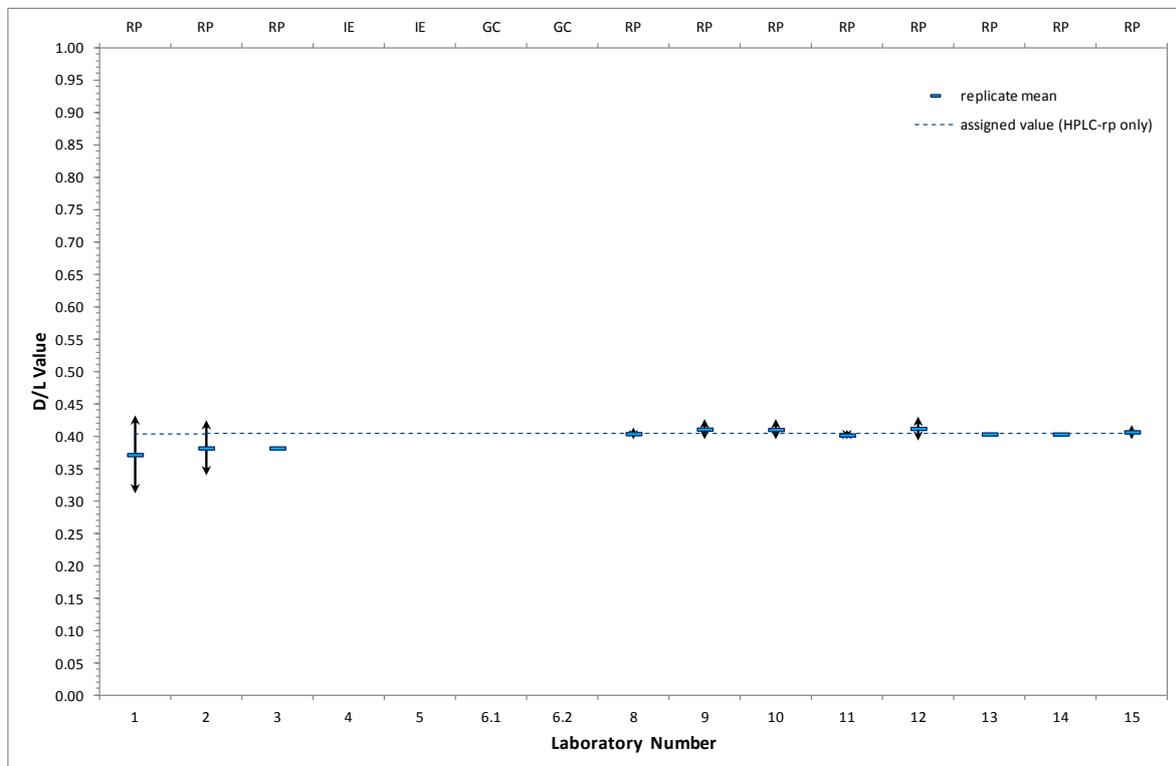


Figure 6.6: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Glutamic acid / Glutamine** D/L Values in Mollusc Shell (B) Test Material

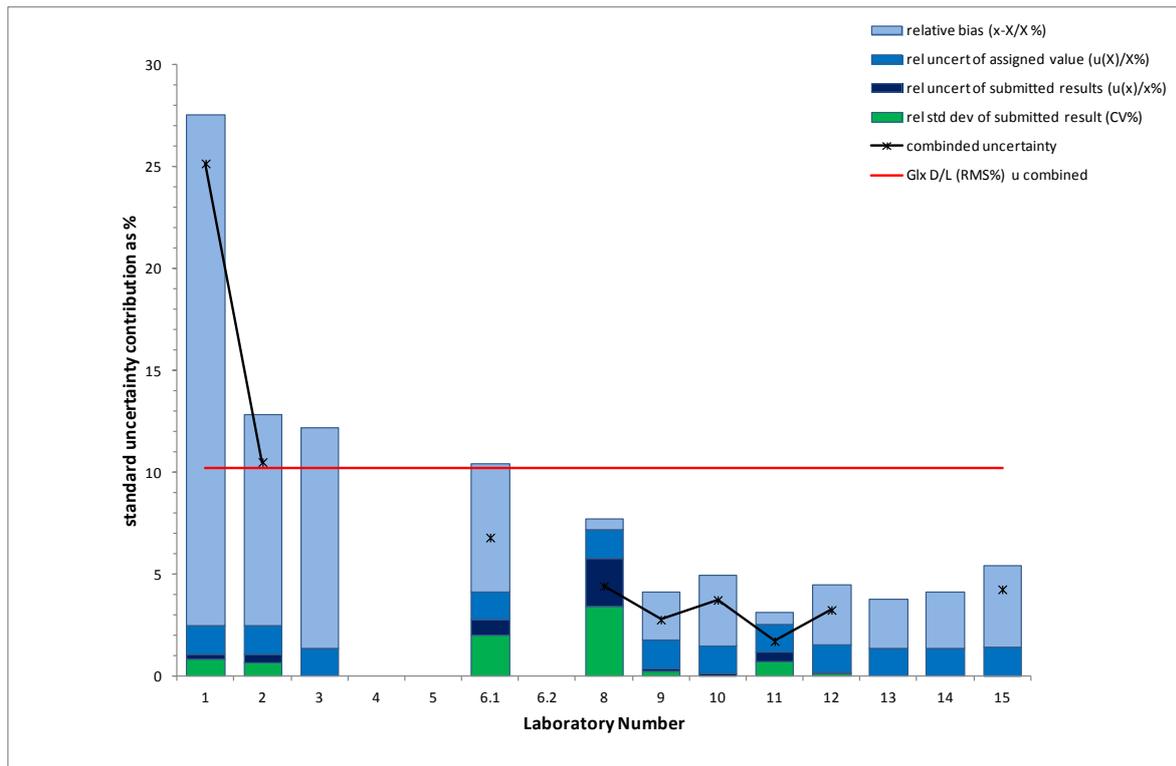


Figure 6.7: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Glutamic acid / Glutamine** D/L Values in Mollusc Shell (B) Test Material

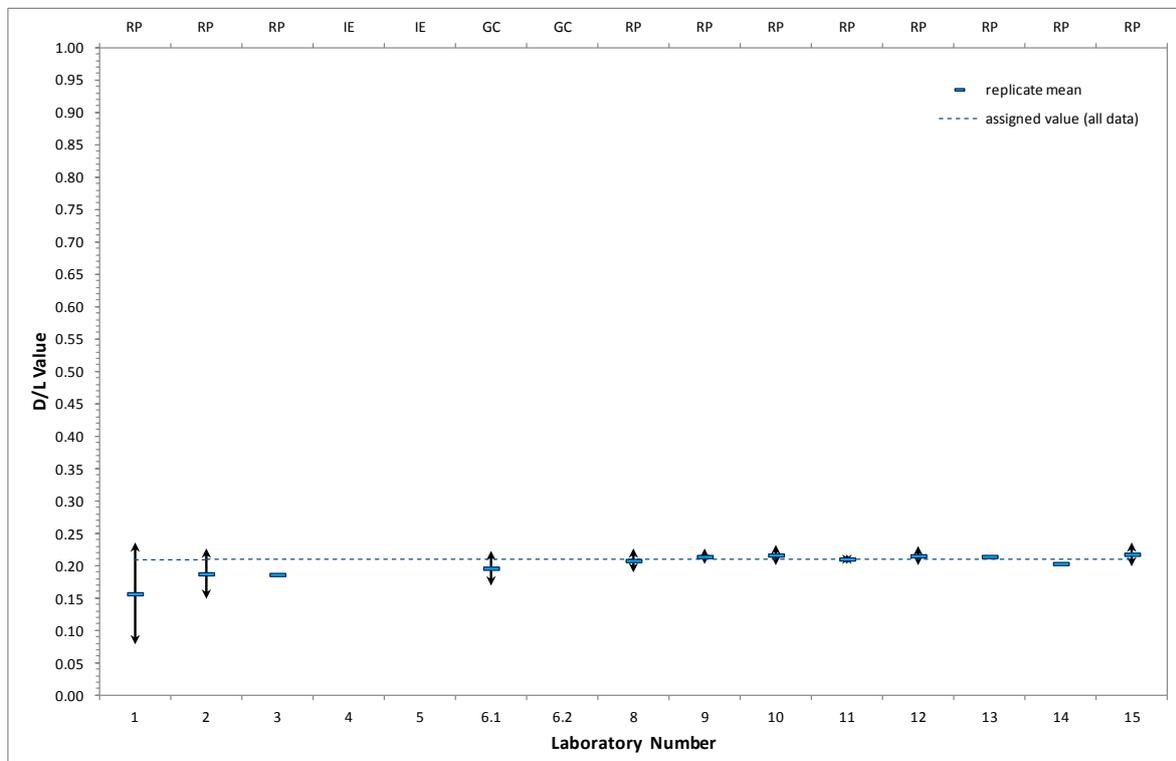


Figure 6.8: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Glutamic acid /Glutamine rpHPLC D/L Values** in Mollusc Shell (B) Test Material

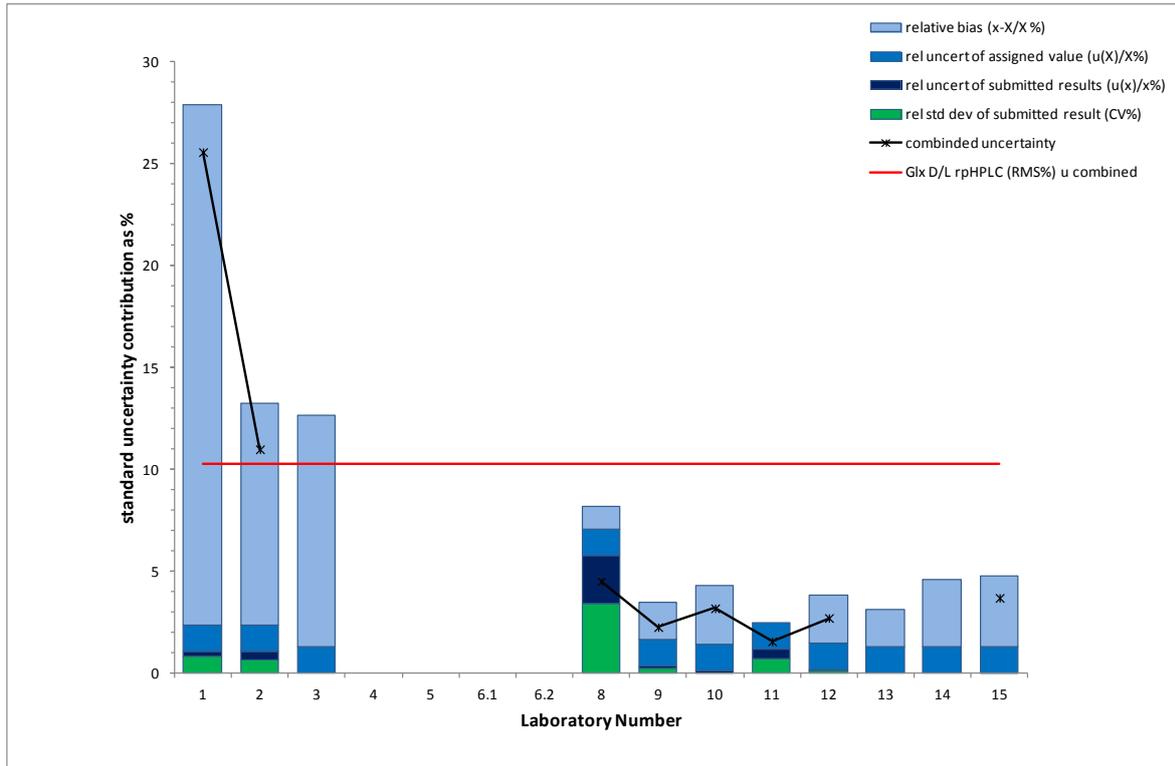


Figure 6.9: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Glutamic acid / Glutamine rpHPLC D/L Values** in Mollusc Shell (B) Test Material

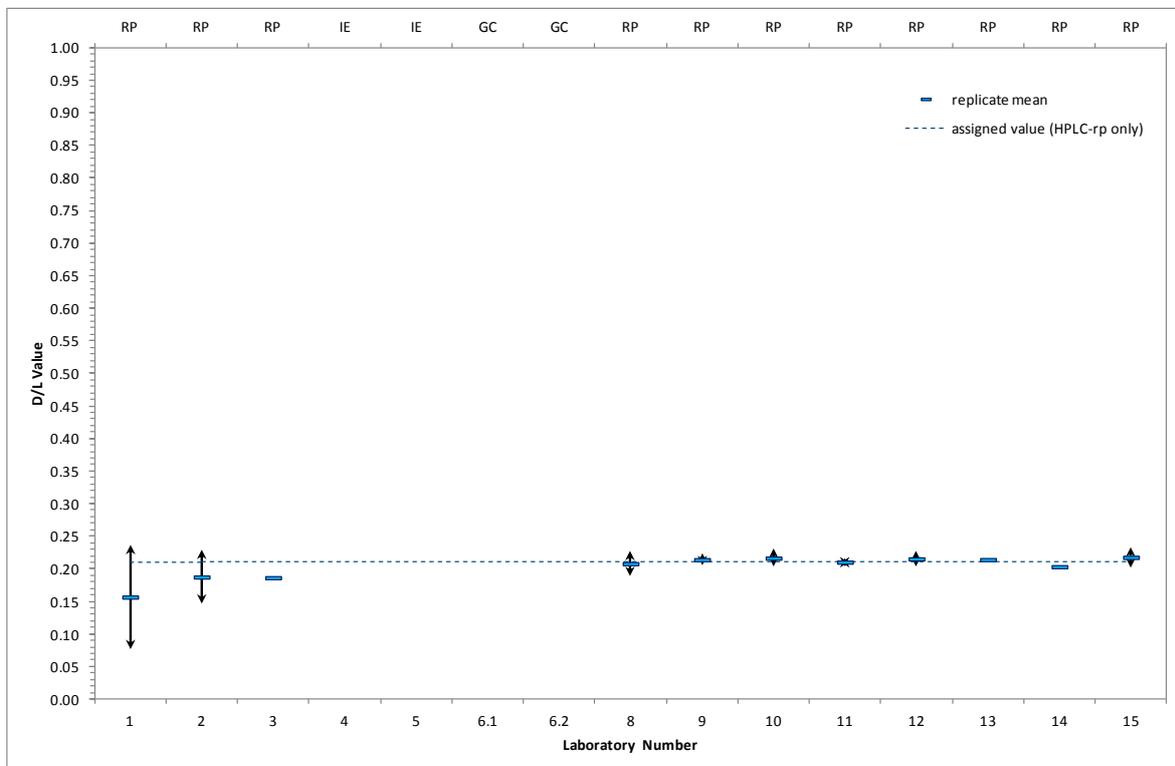


Figure 6.10: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Serine D/L** Values in Mollusc Shell (B) Test Material

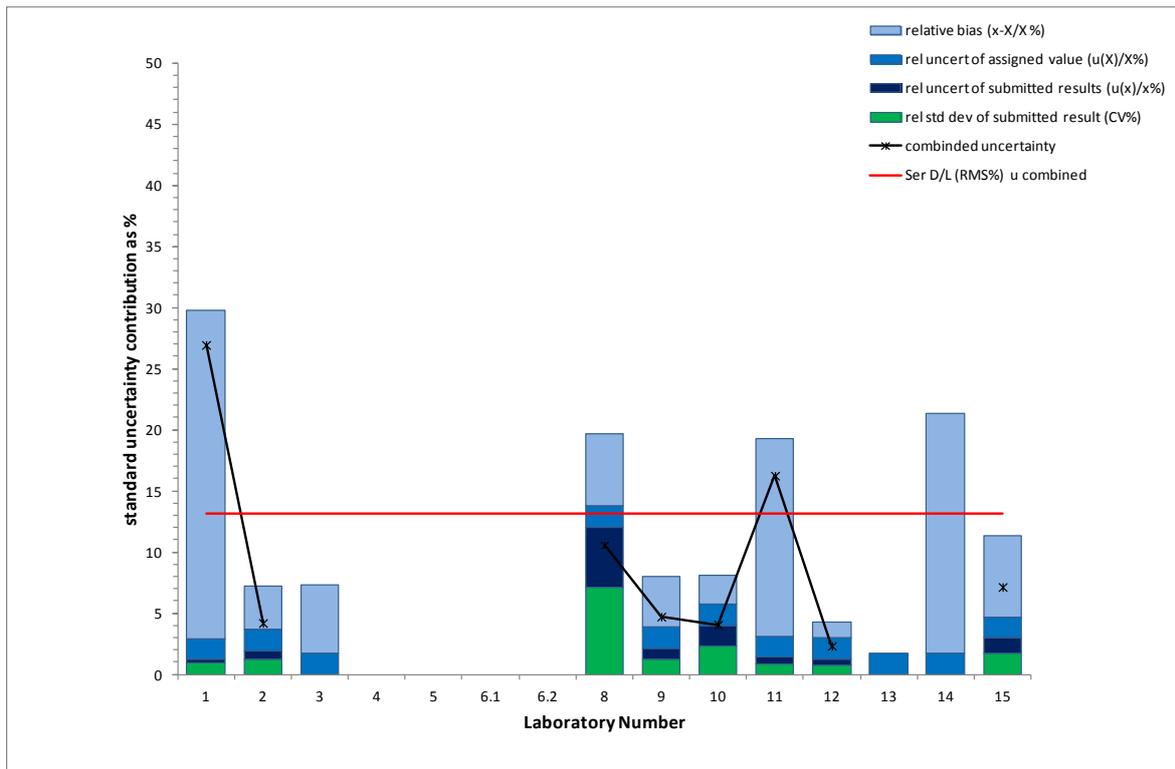


Figure 6.11: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Serine D/L** Values in Mollusc Shell (B) Test Material

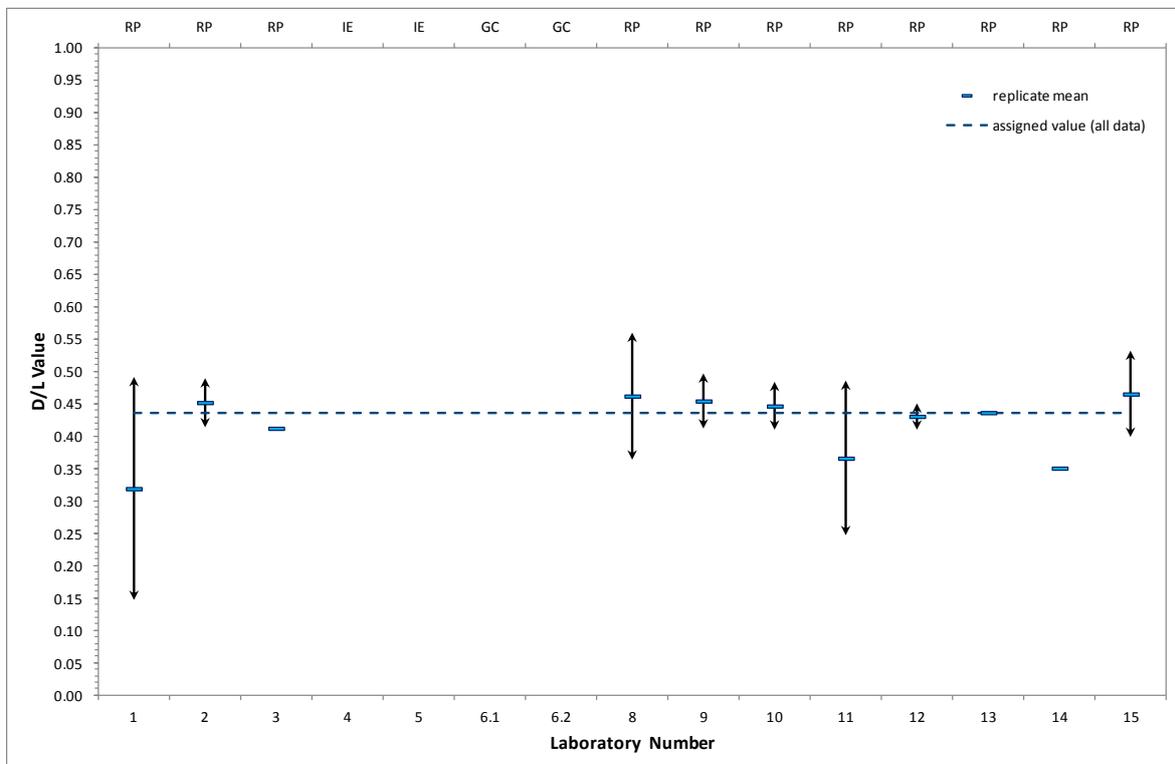


Figure 6.12: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for Arginine D/L Values in Mollusc Shell (B) Test Material

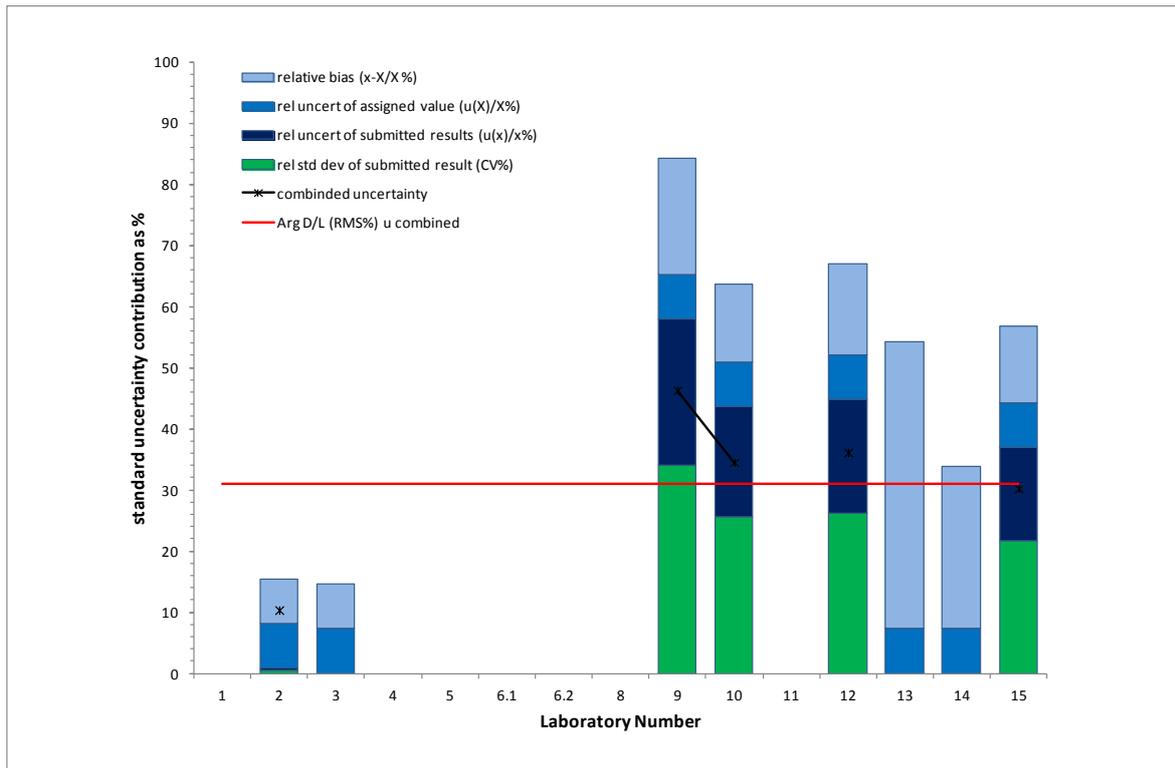


Figure 6.13: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on Arginine D/L Values in Mollusc Shell (B) Test Material

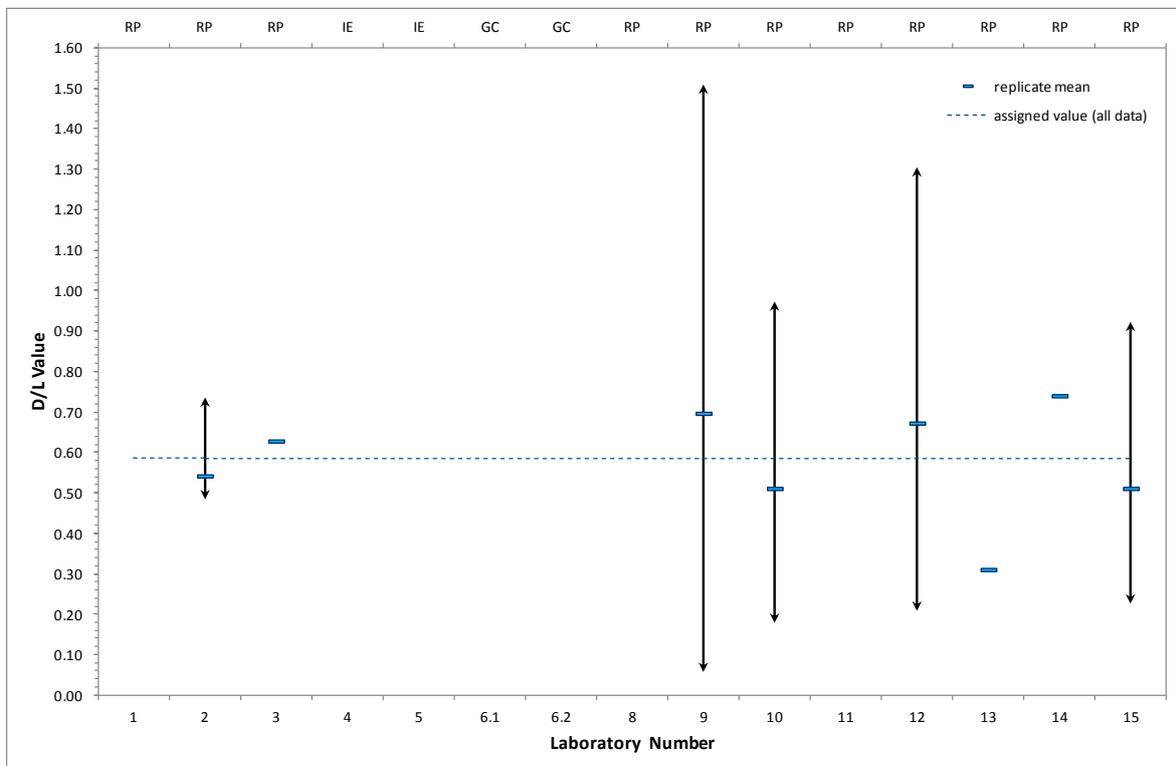


Figure 6.14: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for Alanine D/L Values in Mollusc Shell (B) Test Material

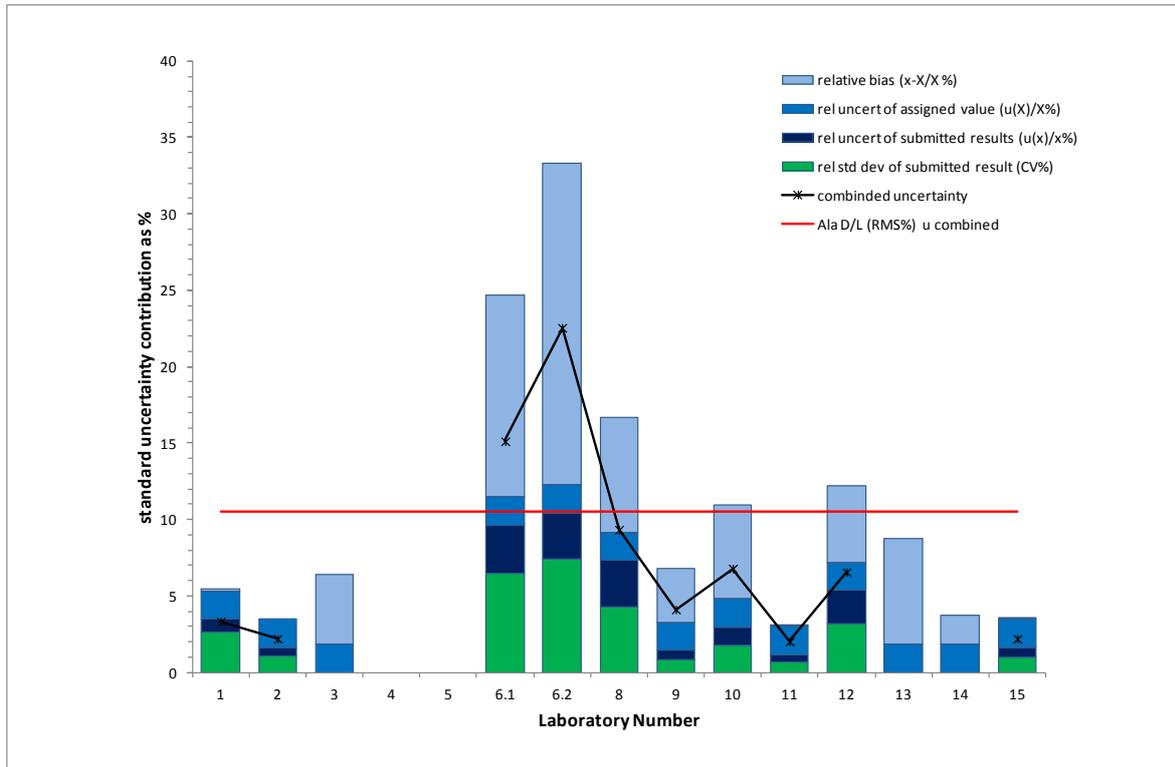


Figure 6.15: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on Alanine D/L Values in Mollusc Shell (B) Test Material

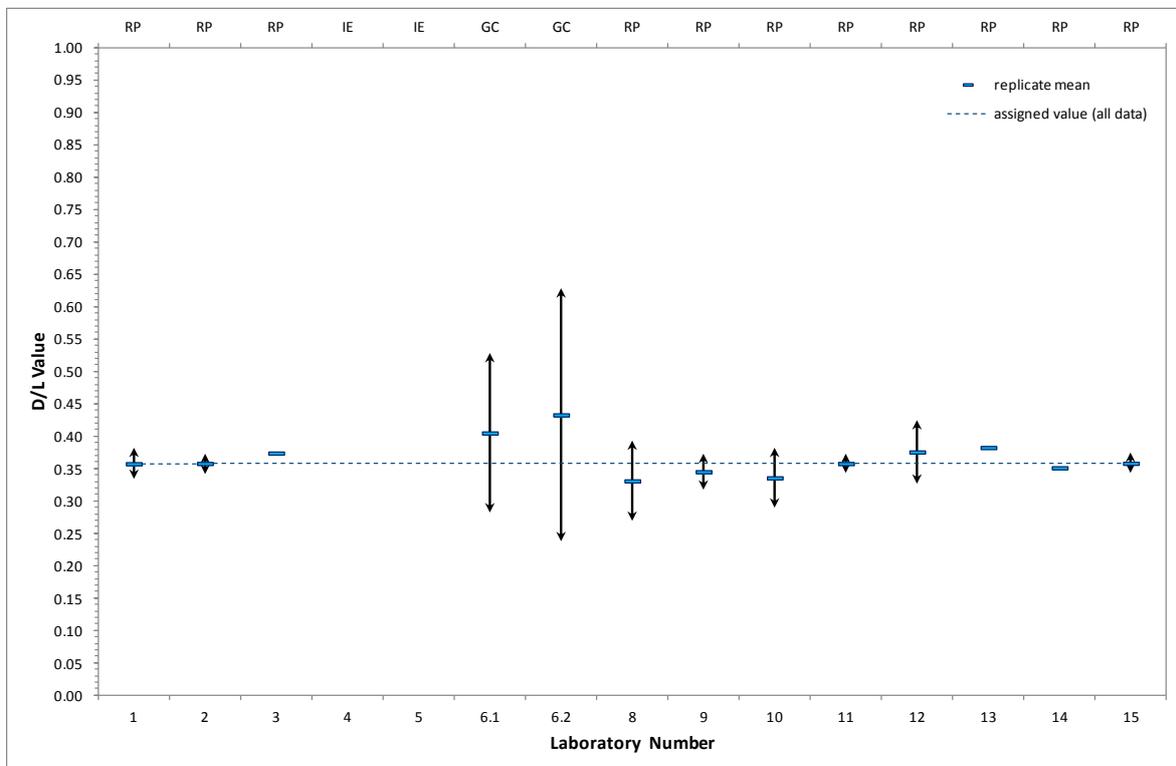


Figure 6.16: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for Alanine (rpHPLC) D/L Values in Mollusc Shell (B) Test Material

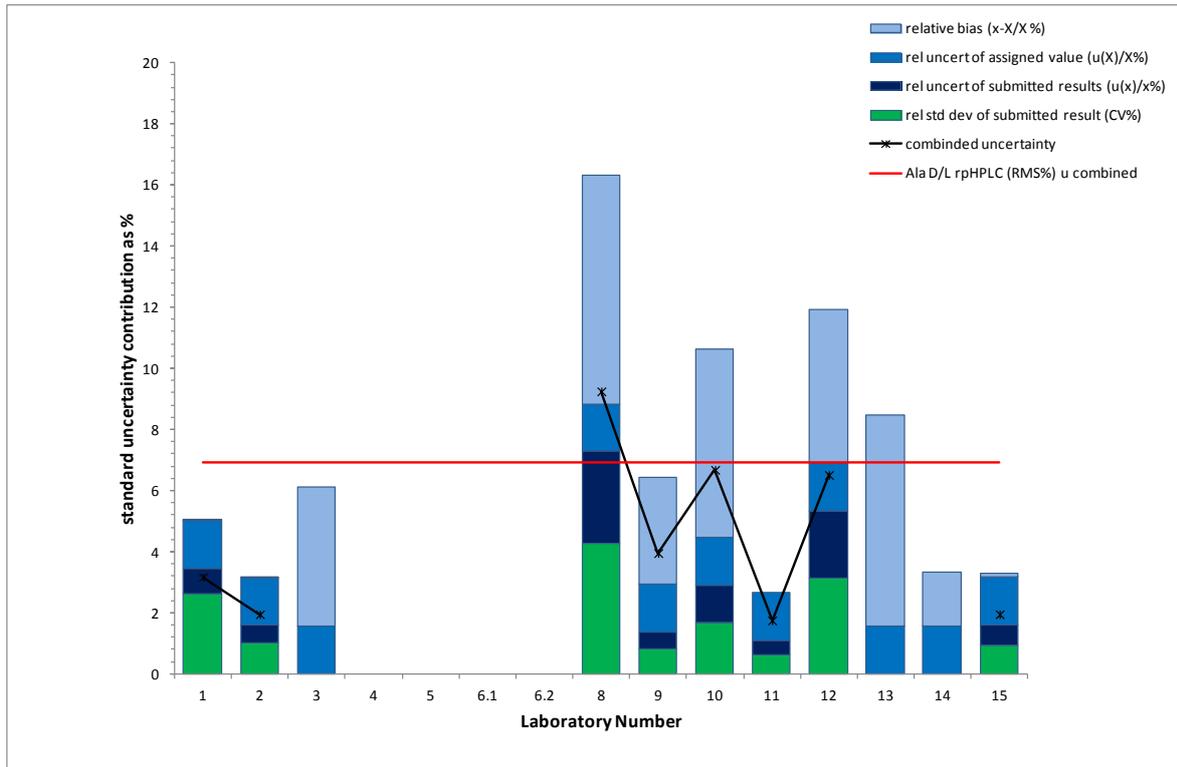


Figure 6.17: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on Alanine (rpHPLC) D/L Values in Mollusc Shell (B) Test Material

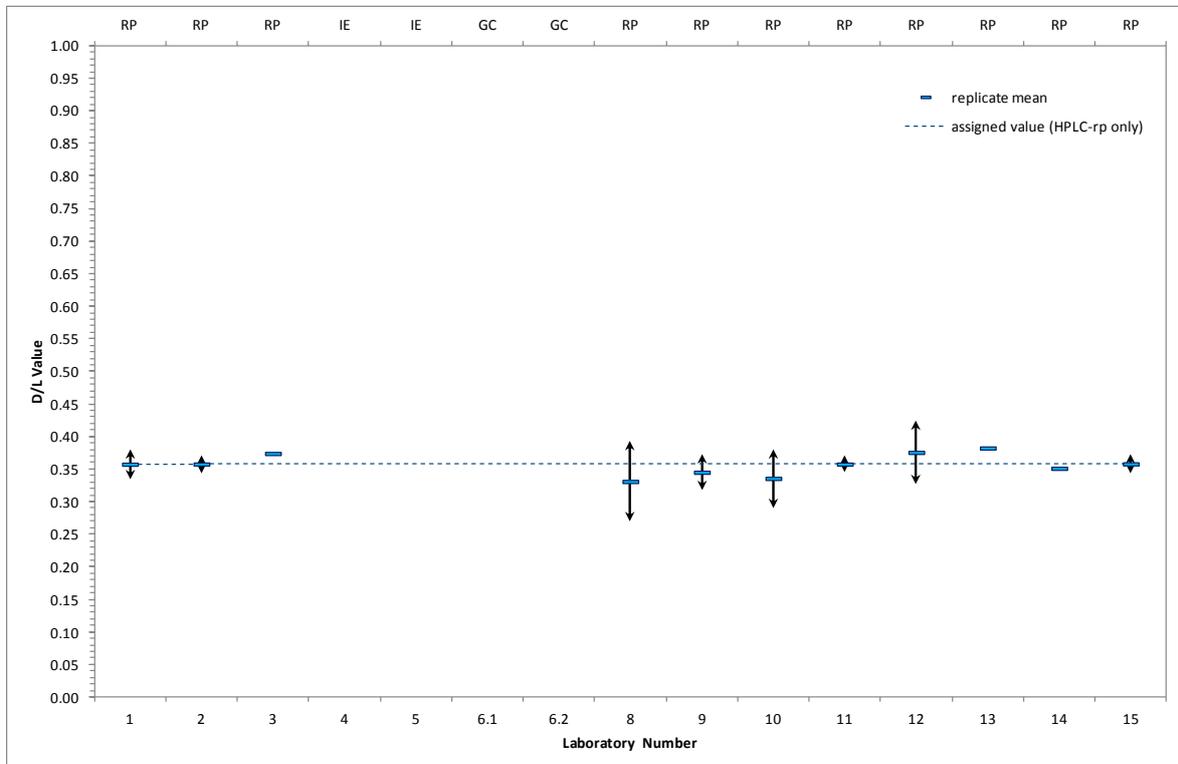


Figure 6.18: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Valine** D/L Values in Mollusc Shell (B) Test Material

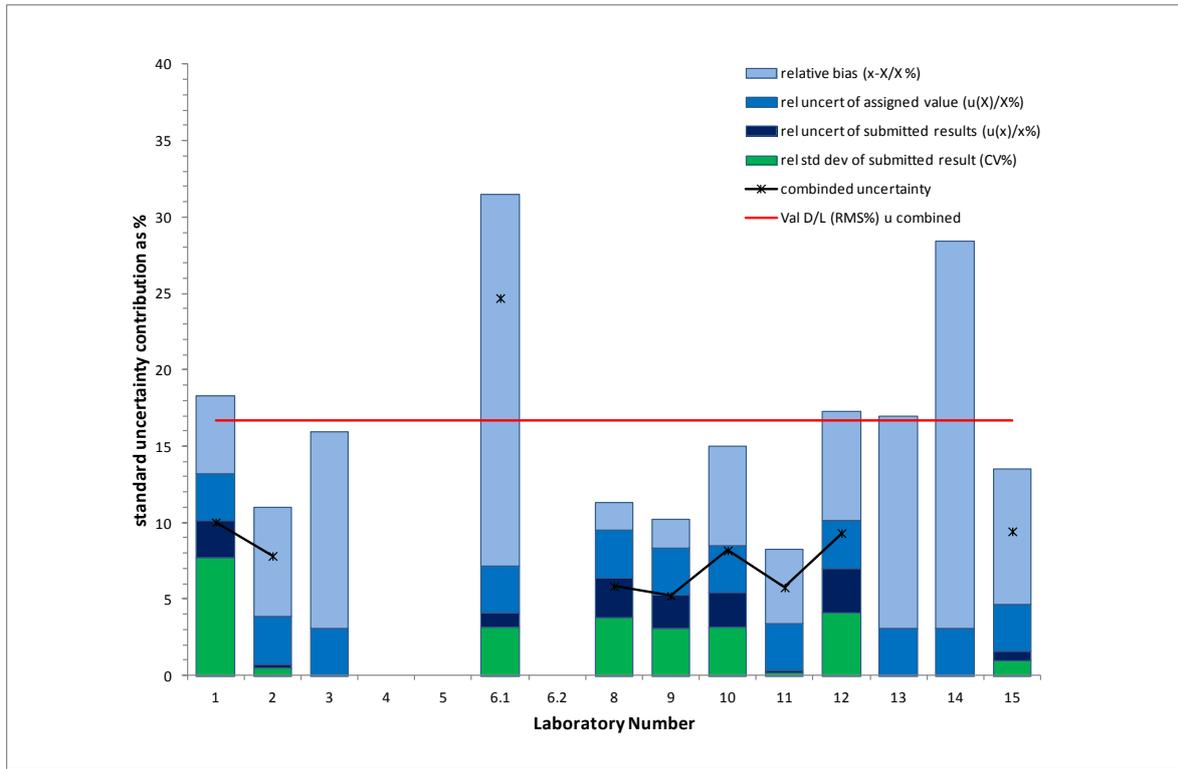


Figure 6.19: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Valine** D/L Values in Mollusc Shell (B) Test Material

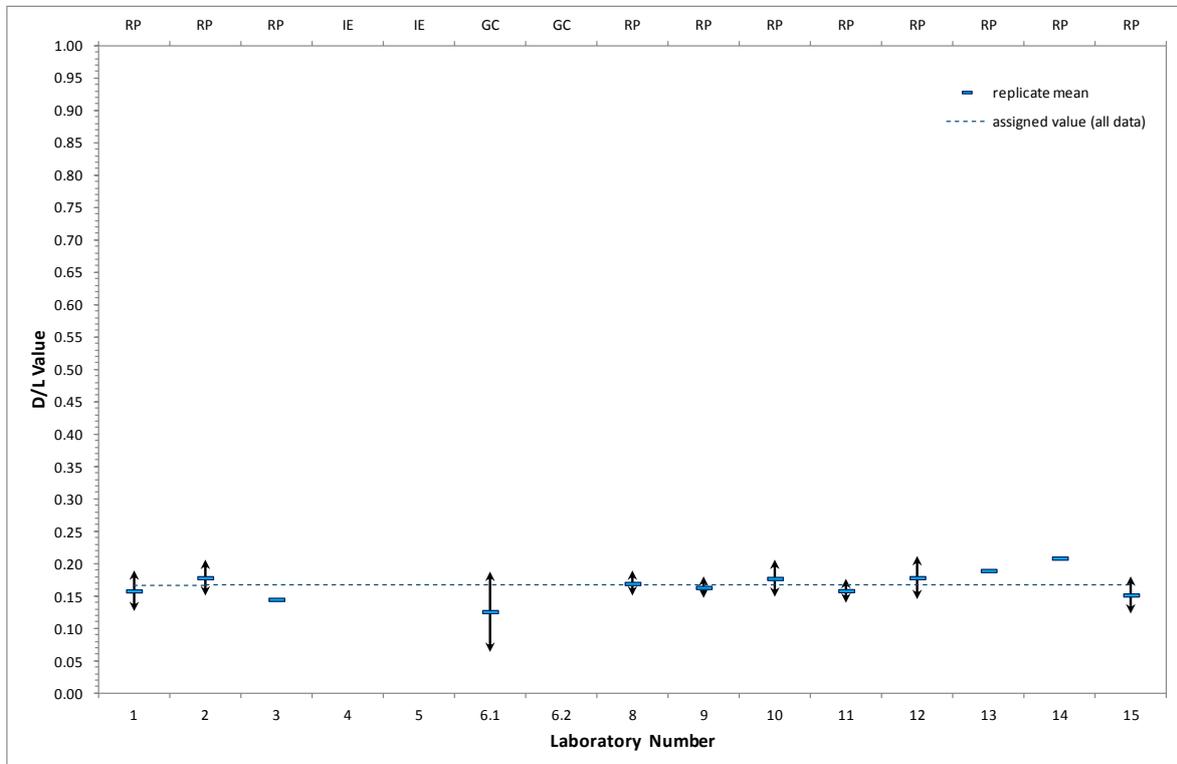


Figure 6.20: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Valine (rpHPLC) D/L Values** in Mollusc Shell (B) Test Material

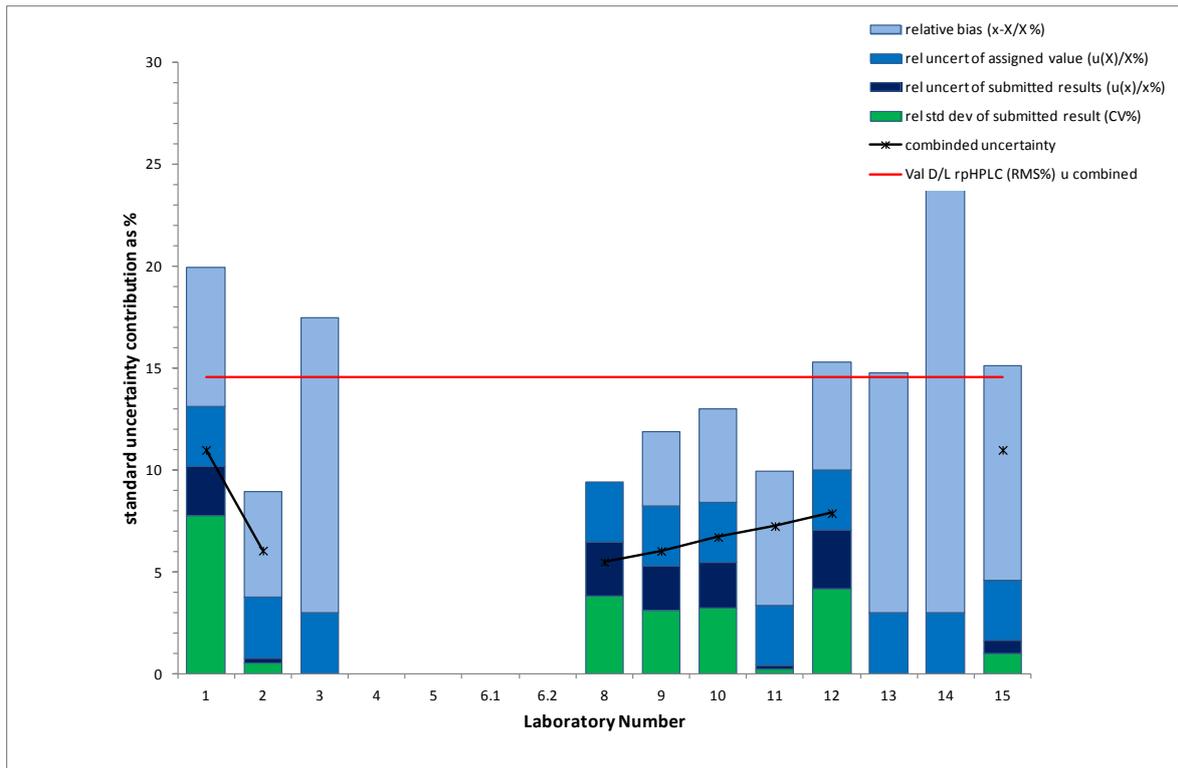


Figure 6.21: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Valine (rpHPLC) D/L Values** in Mollusc Shell (B) Test Material

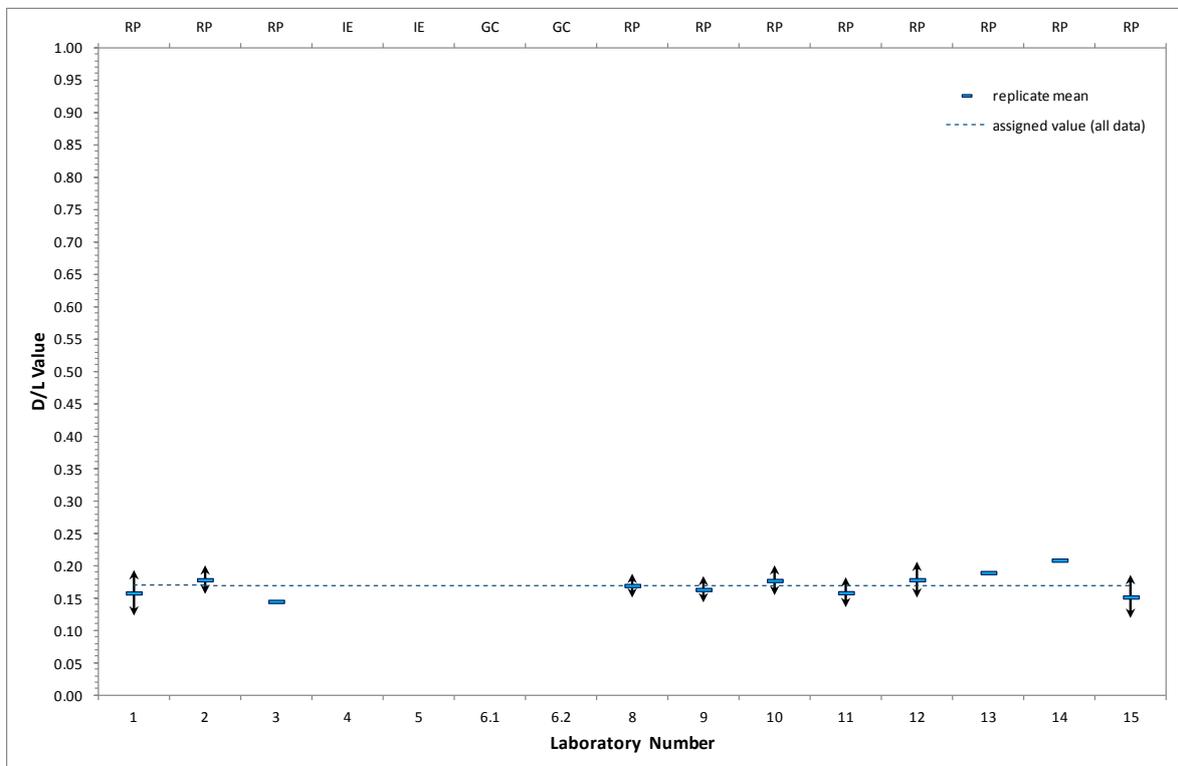


Figure 6.22: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Phenylalanine D/L** Values in Mollusc Shell (B) Test Material

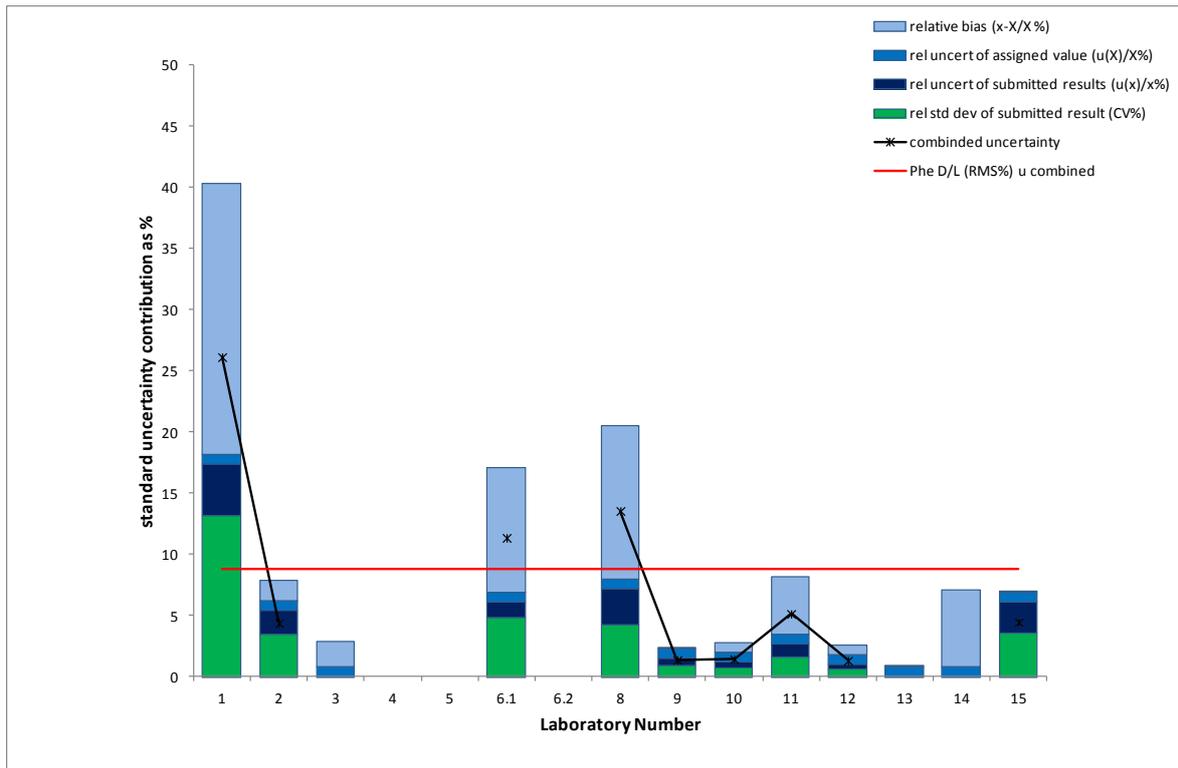


Figure 6.23: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Phenylalanine D/L** Values in Mollusc Shell (B) Test Material

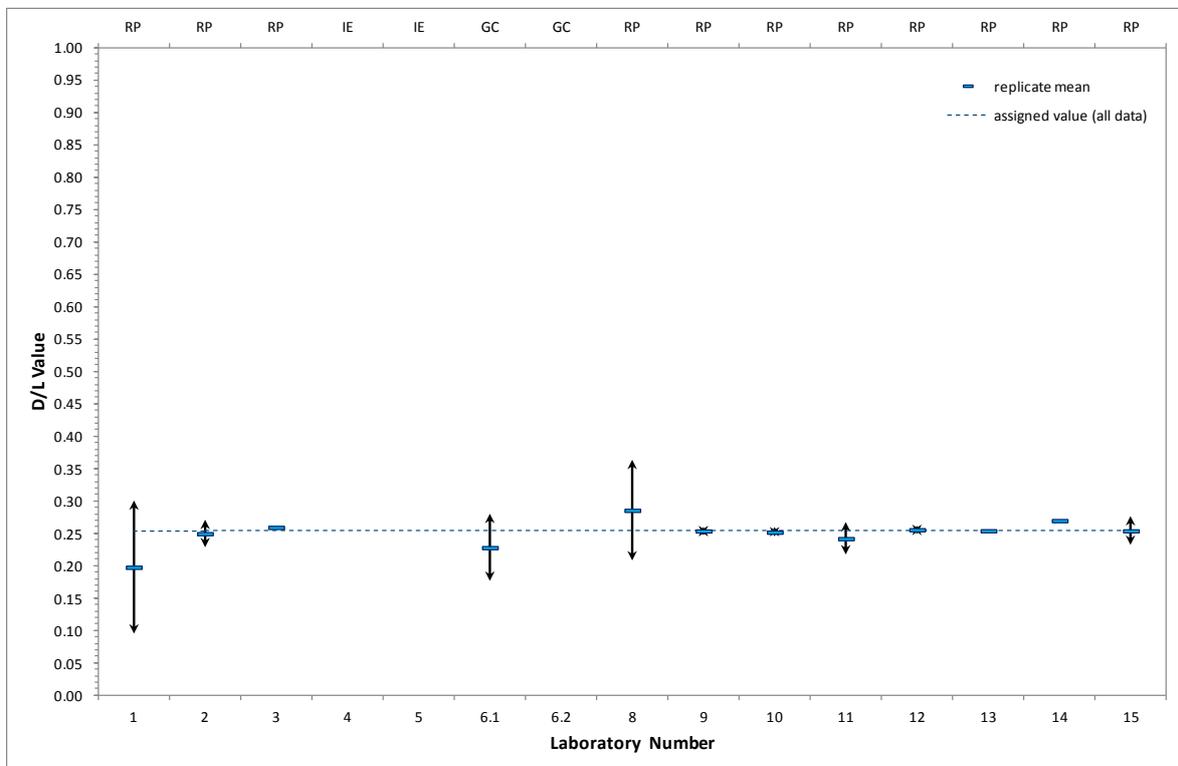


Figure 6.24: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Phenylalanine (rpHPLC) D/L Values** in Mollusc Shell (B) Test Material

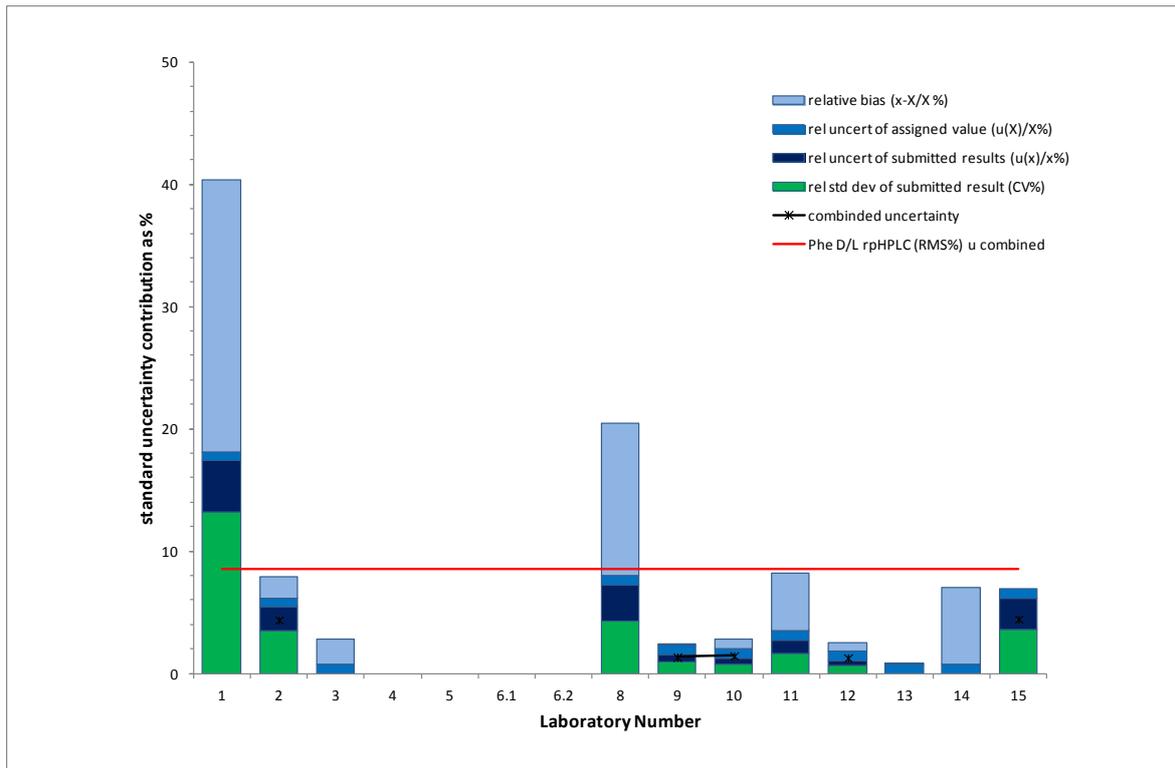


Figure 6.25: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Phenylalanine (rpHPLC) D/L Values** in Mollusc Shell (B) Test Material

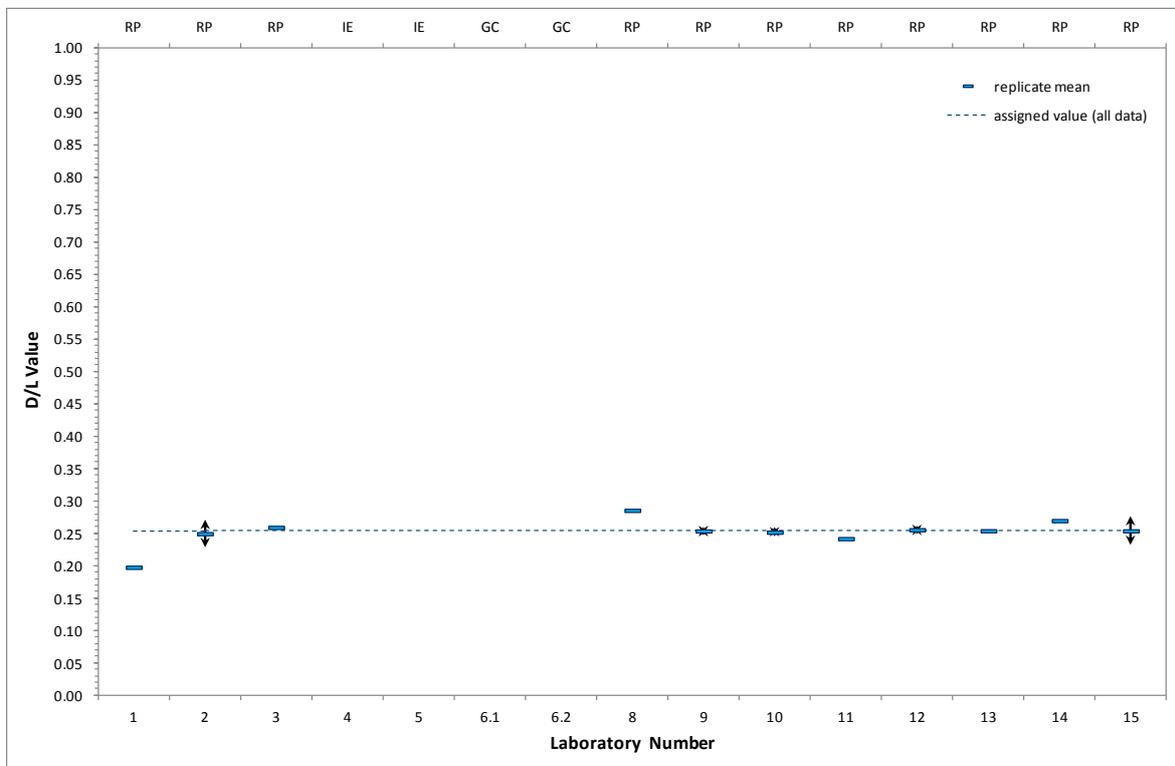


Figure 6.26: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for D-Alloisoleucine/L-Isoleucine Values in Mollusc Shell (B) Test Material

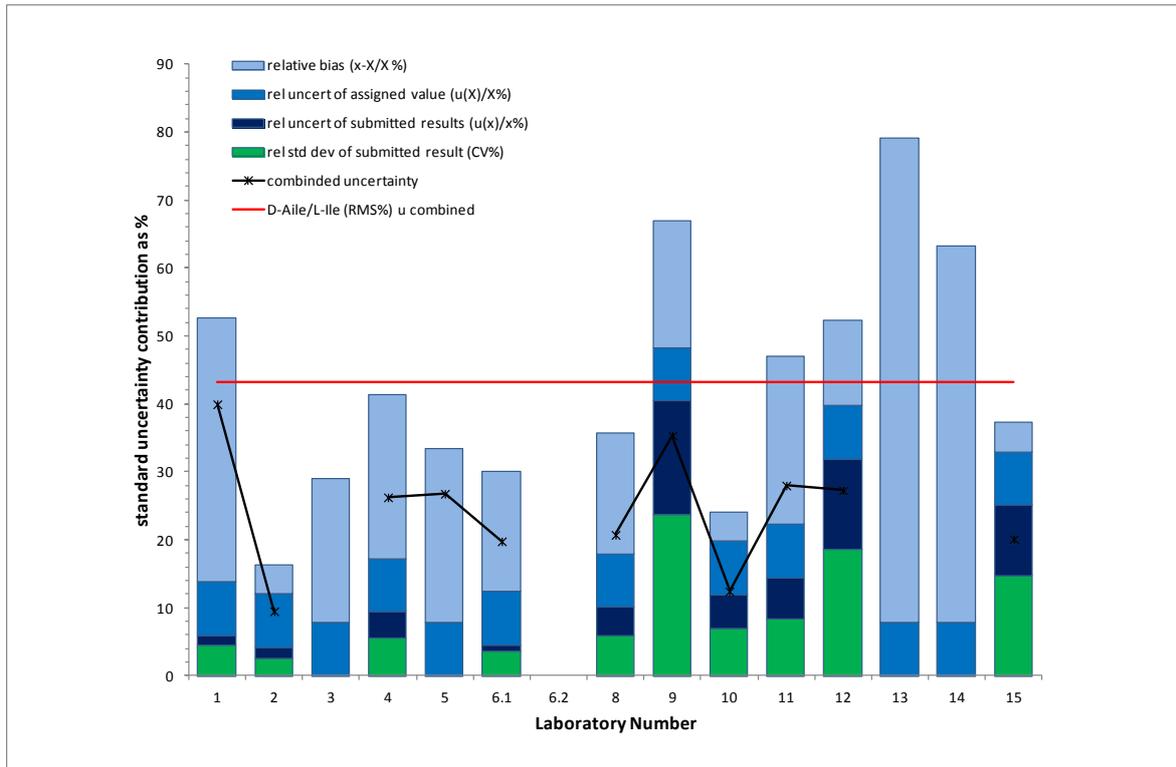


Figure 6.27: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on D-Alloisoleucine/L-Isoleucine Values in Mollusc Shell (B) Test Material

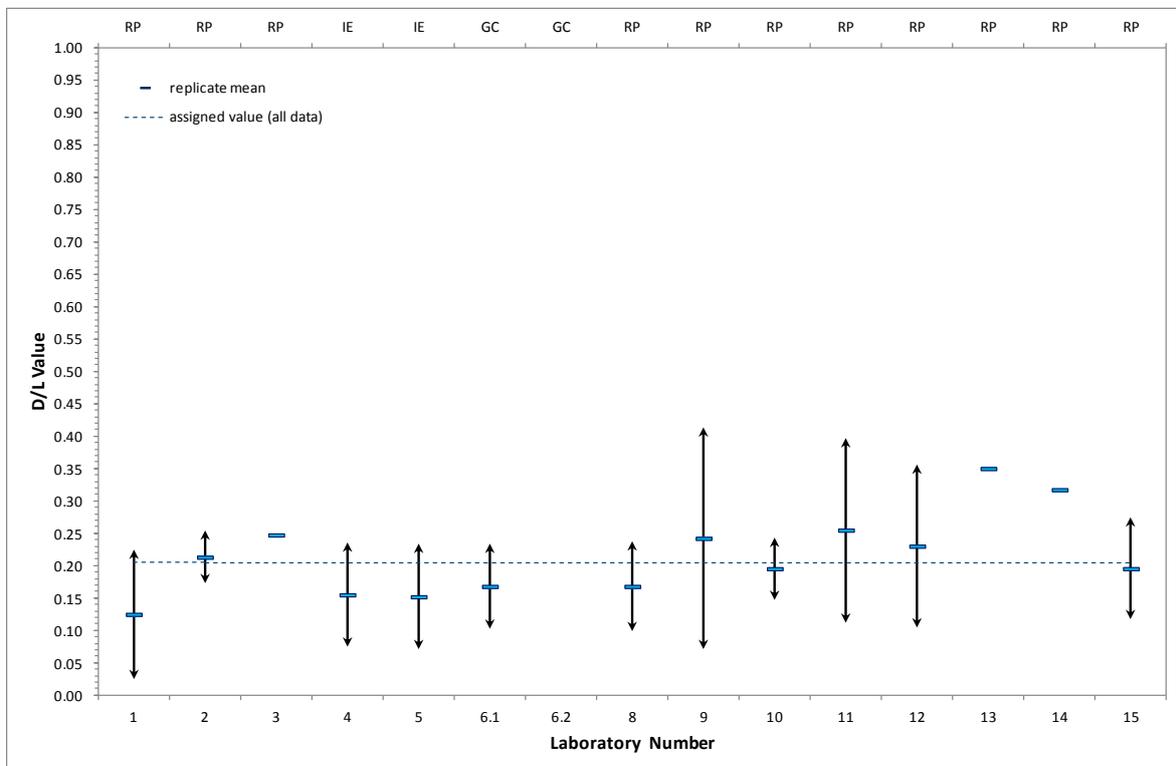


Figure 6.28: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **D-Alloisoleucine/L-Isoleucine (rpHPLC) D/L Values** in Mollusc Shell (B) Test Material

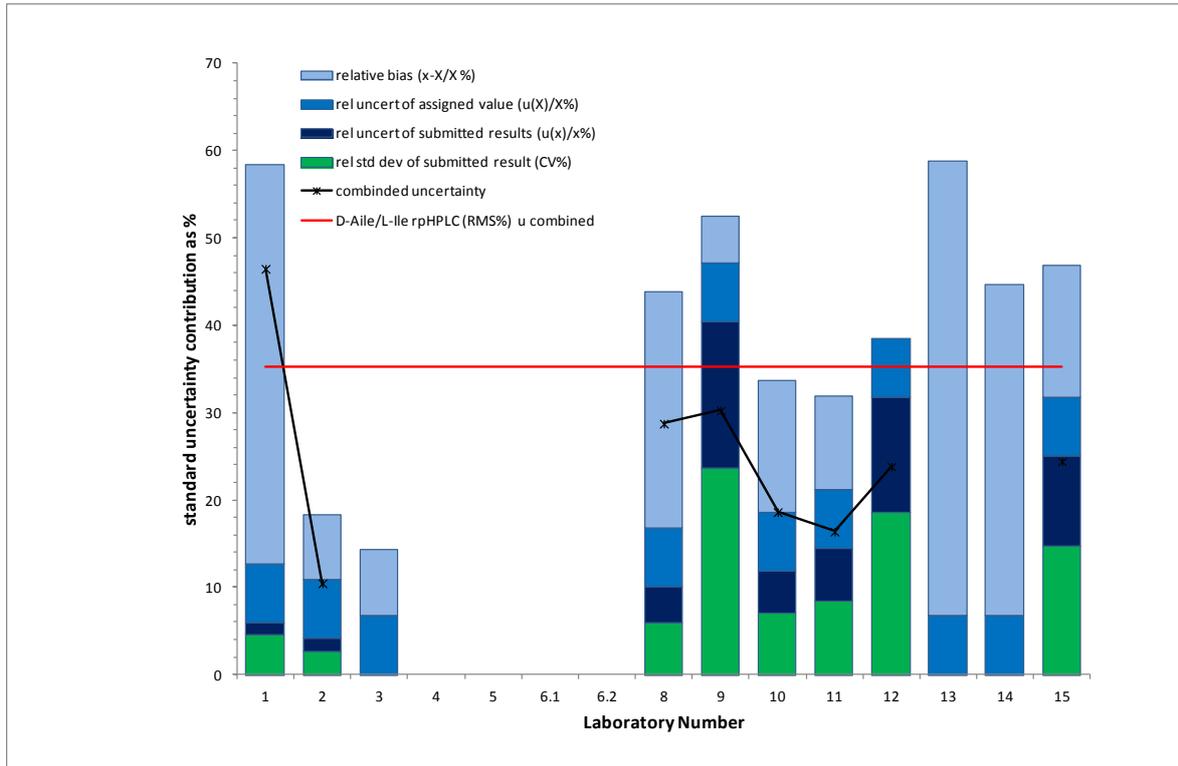


Figure 6.29: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **D-Alloisoleucine/L-Isoleucine rpHPLC Values** in Mollusc Shell (B) Test Material

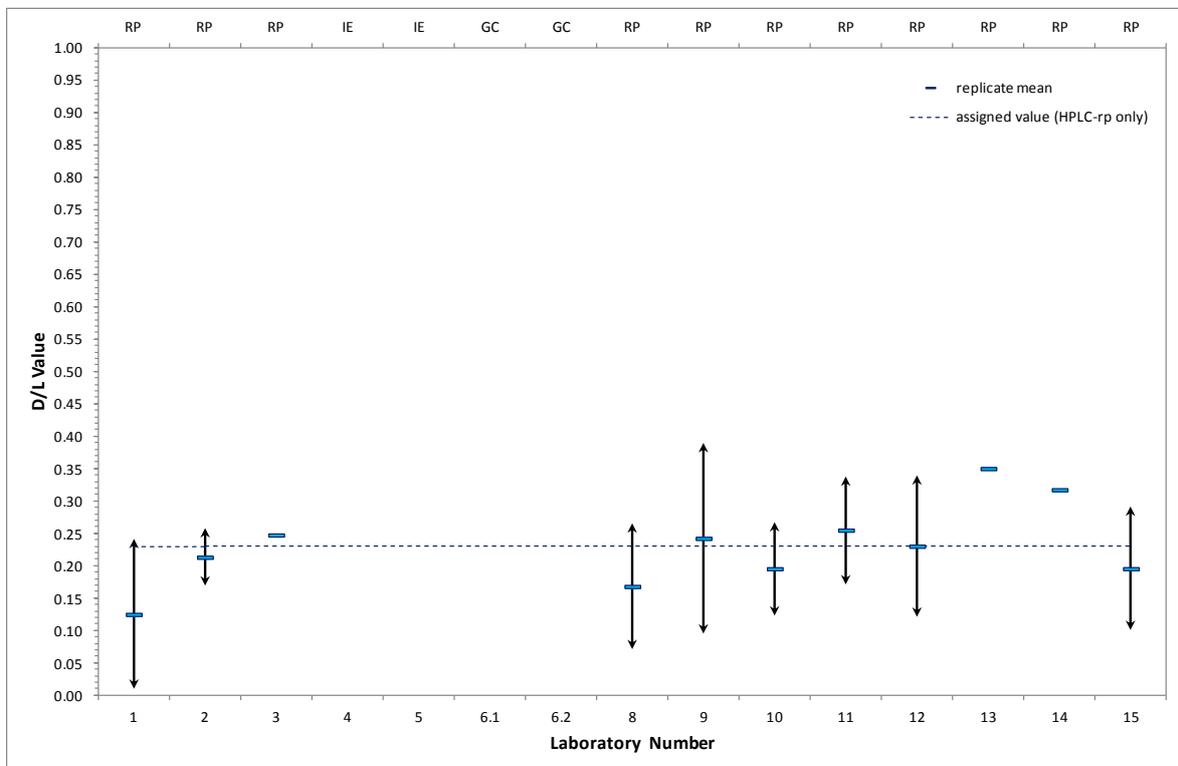


Figure 6.30: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Leucine** D/L Values in Mollusc Shell (B) Test Material

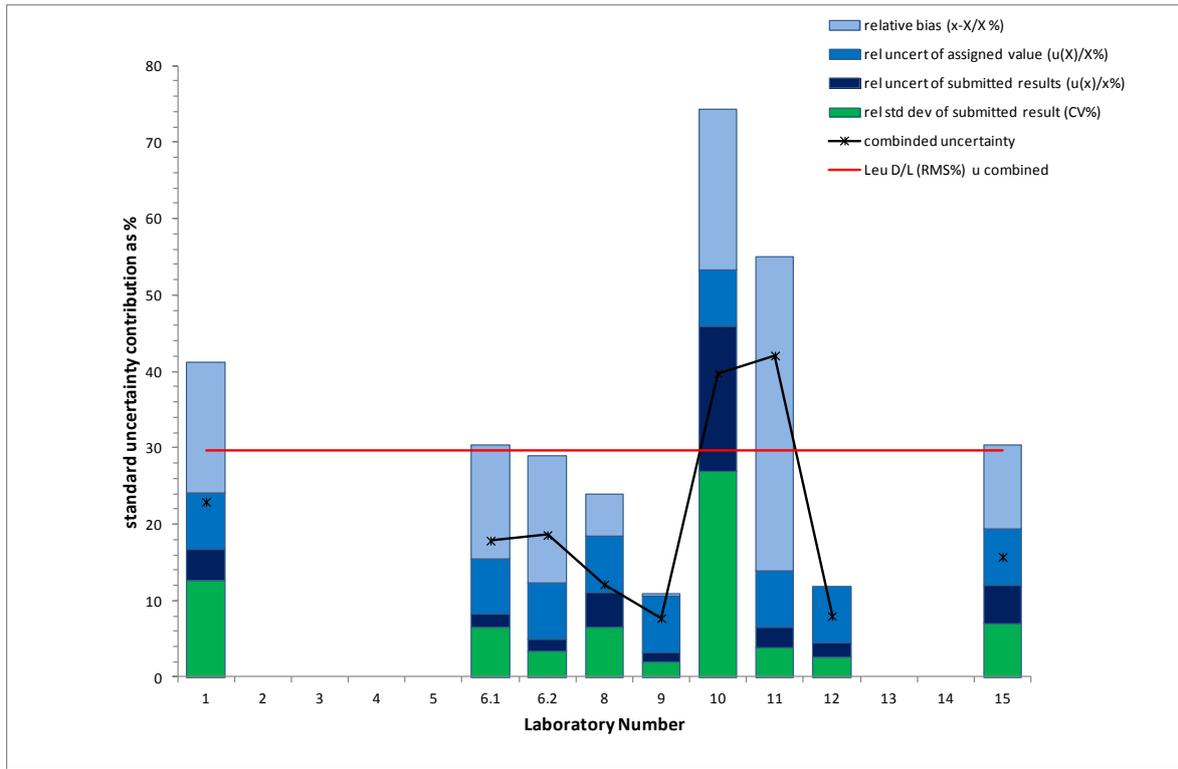


Figure 6.31: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Leucine** D/L Values in Mollusc Shell (B) Test Material

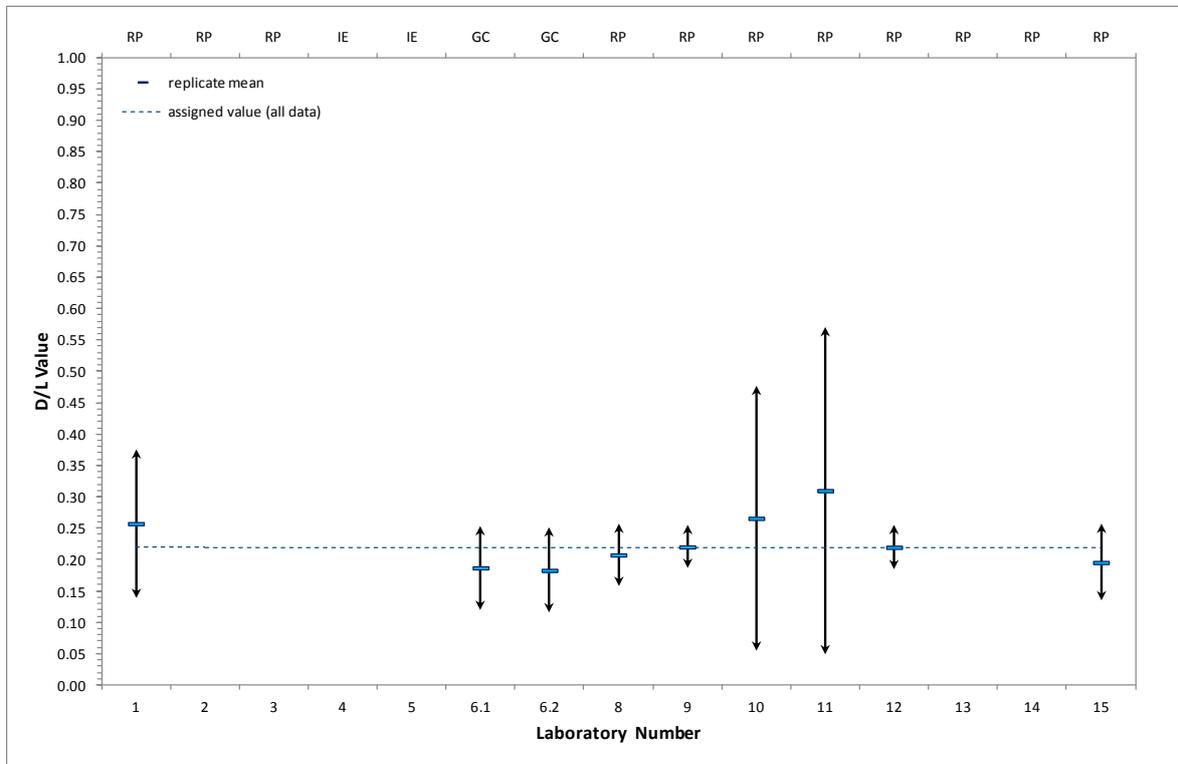


Figure 6.32: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Leucine rpHPLC D/L** Values in Mollusc Shell (B) Test Material

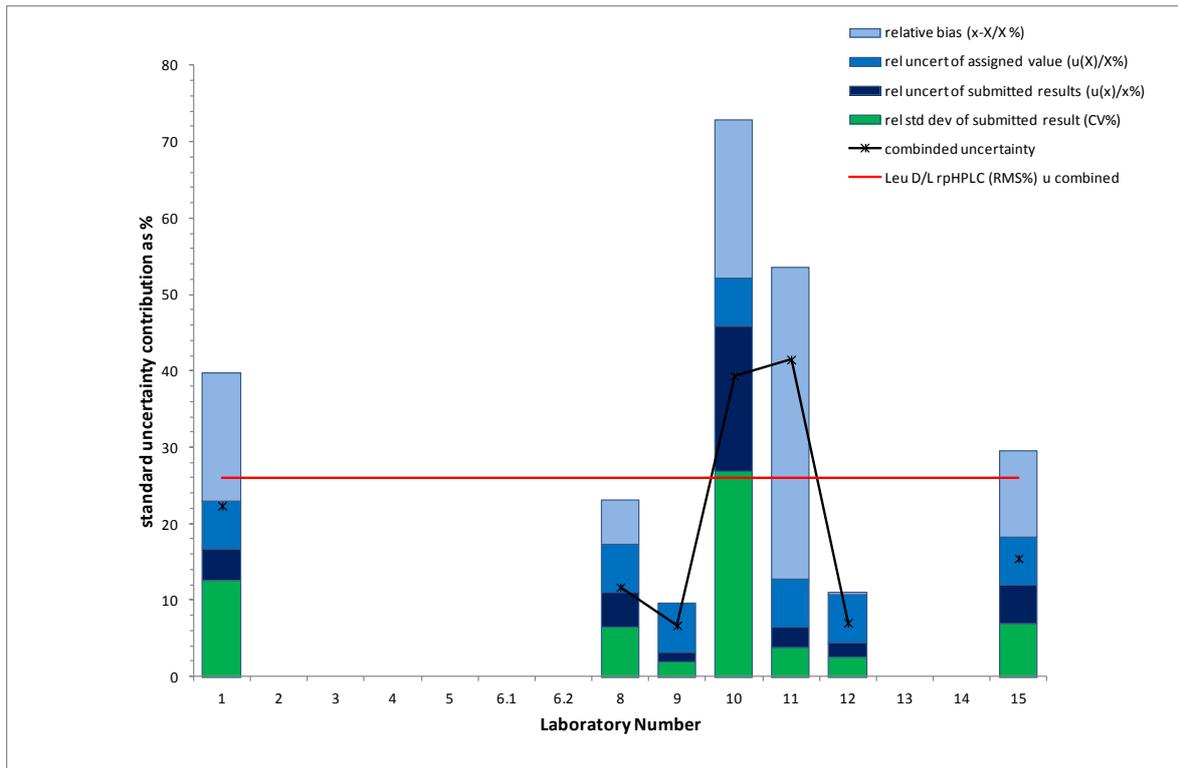


Figure 6.33: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Leucine rpHPLC D/L** Values in Mollusc Shell (B) Test Material

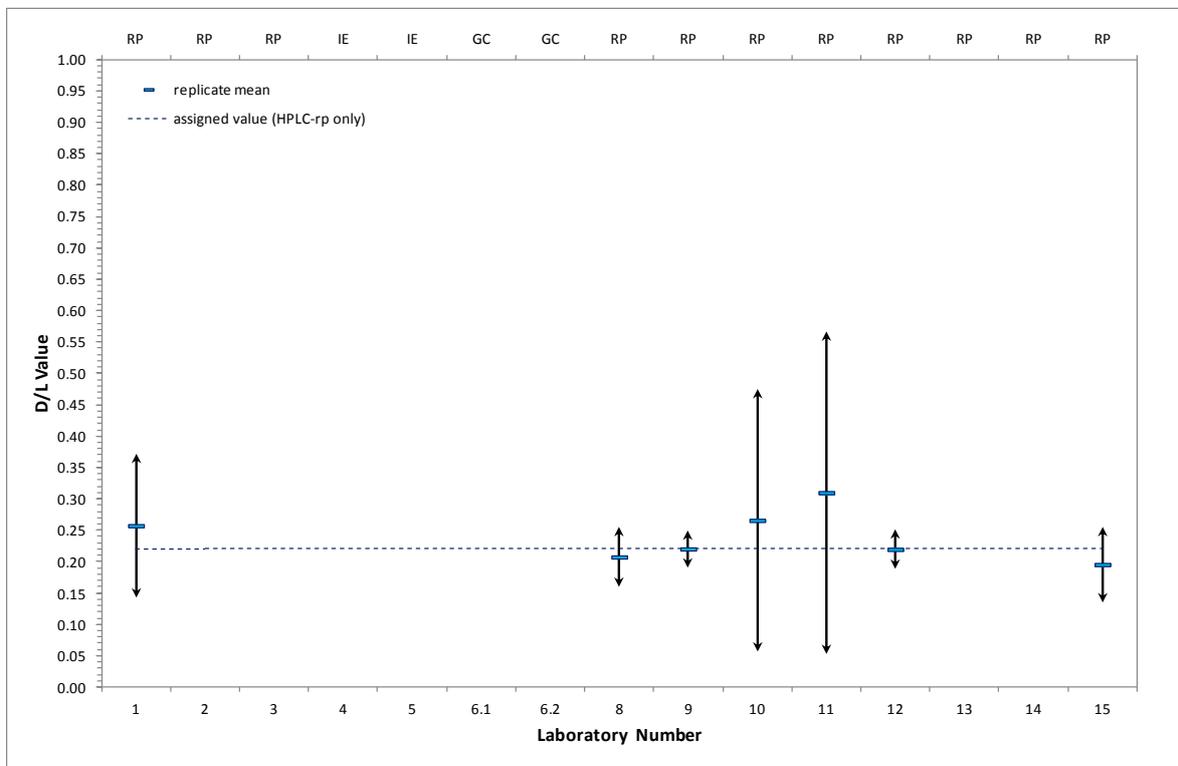


Figure 6.34: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for Tyrosine D/L Values in Mollusc Shell (B) Test Material

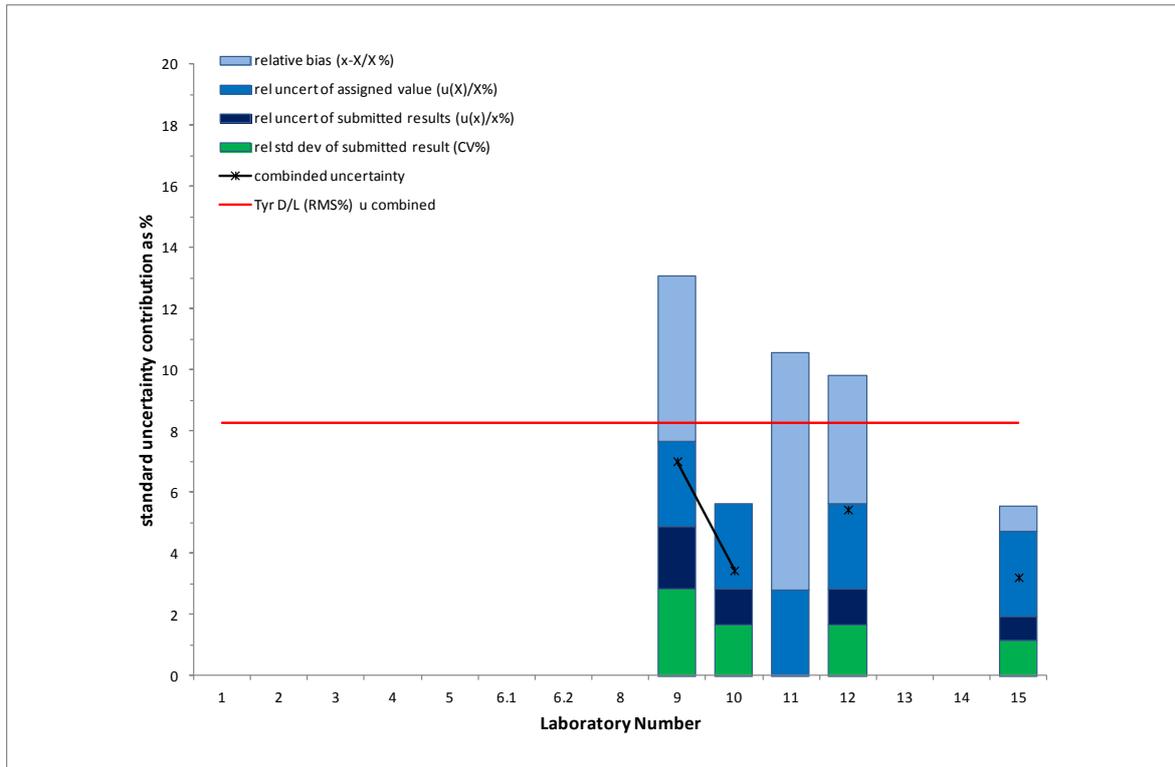
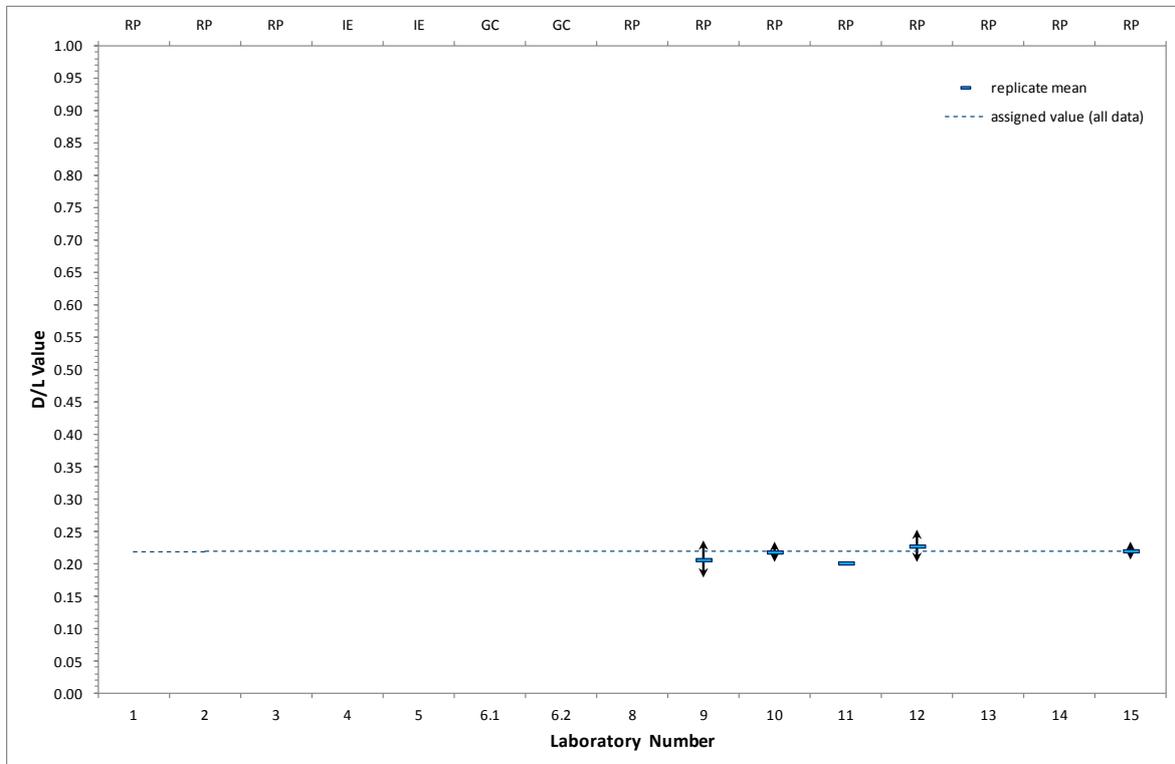


Figure 6.35: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on Tyrosine D/L Values in Mollusc Shell (B) Test Material



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	003
1 – 10 mg	008, 009, 010, 011, 012, 013, 014, 015
>10 – 20 mg	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	001, 009, 010, 011, 012, 013, 014, 015
2)2ml hydrolysis vials used	009, 010, 011, 012, 013, 014, 015
3)samples weighed & transferred to microvial or 4ml vial depending on size.	002, 003, 004, 005
Oven Temperature (°C):	
100 °C	001
110 °C	009, 010, 011, 012, 013, 014, 015
Heating Time (hours):	
6 hrs	002, 003
20 hrs	001
22 hrs	004, 005, 008
24 hrs	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	001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015
Ambient / room temp	001, 002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015
Dried overnight	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	001
20 µl/mg	002, 003, 004, 005, 008, 009, 010, 011, 012, 013, 014, 015
Internal Standard Used?:	
L-homo-Arginine	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
Norleucine	004, 005
Concentration of Internal std used (M):	
0.03 mM	001
0.01mM	002, 003, 008, 009, 010, 011, 012, 013, 014, 015
6.25 mM	004, 005
Source / supplier of internal standard:	
Sigma	001, 002, 003, 004, 005
Sigma Aldrich (Fluka)	008
Other constituents and their concentrations (M or mM) in rehydration fluid:	
0.01M HCl	002, 003, 004, 005, 009, 010, 011, 012, 013, 014, 015
1.5mM Sodium Azide	009, 010, 011, 012, 013, 014, 015
ANALYSIS	
Please state method used	
Reverse phase HPLC	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
Ion Exchange HPLC	004, 005
Instrument used	
Agilent 1100 Series	001, 008, 009, 012, 013
Agilent / Hewlet Packard 1100 Series	002, 003, 010, 011, 014, 015
Agilent 1200 Series	004, 005
Agilent 6890 GC, Flame Ionization	006, 007
Pre-column Derivatization Reagent constituents and their concentrations (M or mM):	
OPA 170 mM	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
IBLC 260 mM	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
Potassium borate buffer 1M	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	001, 002, 003, 009, 010, 011, 012, 013, 014, 015
4 µl	008
20 µl	004, 005

HPLC COLUMN	
Column Make/Type & Phase(i.e.; Hypersil BDS)/ Batch Number:	
Thermo/Hypersil BDS C18/0742018X Hypersil BDS Hypersil BDS /5/120/4772 Pickering Labs Sodium Cation Exchange Supelcosil LC-18-DB(rp)/6520/5-1452	001 009, 010, 011, 012, 013, 014, 015 002, 003 004, 005 008
Column Packing:	
Silica Sodium Functional group; C ₁₈ End capped (Yes)	002, 003, 008 004, 005 001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015 002, 003, 008
Column width (mm)	
3mm 5mm	001, 002, 003, 004, 005 009, 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 30 °C	001, 009, 010, 011, 012, 013, 014, 015 002, 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) Methanol Acetonitrile Sodium citrate buffer (pH 3.12) Sodium citrate buffer (pH 3.86) Sodium chloride buffer (pH 11.5)	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, 015 004, 005 004, 005 004, 005
Sodium acetate Buffer (pH 6.00) Gradient: Starting % Final % time (mins) flow rate (ml/min)	
95% 76.6% 31mins 0.56ml/min 76.6% 46.2% 95min 0.60ml/min 95% 5% 83min 0.500ml/min 95% 50% 88min 0.560ml/min 95% % 95min 0.56ml/min	001a 001b 002, 003 008 009, 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	001a
23% 48.8% 95min 0.60ml/min	001b
5% 95% 83min 0.500ml/min	002, 003
5% 45% 88min 0.560ml/min	008
5% 50% 95min 0.56ml/min	009, 010, 011, 012, 013, 014, 015
Acetonitrile Gradient: Starting % Final % time (mins) flow rate (ml/min)	
0% 0.4% 31mins 0.56ml/min	001a
0.4% 5% 95min 0.60ml/min	001b
0.4% 5% 83min 0.500ml/min	002, 003
0% 5% 88min 0.560ml/min	008
0% 5% 95min 0.56ml/min	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	004,005
OPA 0.0075M	004,005
Ethanol 1%	004,005
2-mercapthoethanol 0.00075%	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	008, 009, 010, 011, 012, 013, 014, 015
250	002, 003
335	001
340	004, 005
Emission wavelength (nm):	
410	002, 003
445	001, 008, 009, 010, 011, 012, 013, 014, 015
455	004, 005

Gas Chromatography

REFERENCES	
Please give details of any method relevant references;	
Goodfriend 1991 with modifications	006
HYDROLYSIS FOR THAA's	
Sample Weight used for analysis (mg):	
75 - 90 mg	006
Vials used for hydrolysis:	
Glass	006
Acid Used:	
6M HCl	006
Vials flushed with N ₂ :	
Yes	006
Please give details of any other treatment prior to hydrolysis:	
Comments received (006); Samples weighed into hydrolysis vials without drying; fossil samples are always dried in vacuo prior to weighing for hydrolysis.	
Oven Temperature (°C):	
105 °C	006
Heating Time (hours):	
22 hrs	006
SAMPLE CLEAN UP / DESALTING	
Was cation exchange resin used?	
No	006
Was HF used to separate amino acids from precipitate?	
Yes	006
Was sample dried prior to Derivatization?:	
Yes	006
Please give details of sample drying conditions:	
Under nitrogen stream	006
Drying Temp; 50 °C (in heating block)	006
Drying time; 1 hr	006

SAMPLE CLEAN UP / DESALTING continued	
Comments received (006); After HF removal of Ca, solution of AA was dried under N ₂ 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
Esterification conditions:	
Flushed under nitrogen	006
Oven Temperature; 50°C	006
Heating time; 1hr	006
Was sample dried prior to acylation?:	
Yes	006
Please give details of sample drying conditions:	
Under vacuum	006
Under nitrogen stream	006
Drying Temp; 55 °C	006
Drying time; 1 hr	006
ACYLATION	
Acylation reagents:	
TFAA	006
Acylation conditions:	
Flushed under nitrogen	006
Room Temperature	006
Heating time; 2hr minimum	006
Comments received (006); Isopropanol has to be removed before TFA can be added (with Methylene chloride)	
Was sample dried prior to GC analysis?:	
Yes	006
Please give details of sample drying conditions:	
Flushed under nitrogen	006
Room Temperature	006
Heating time; <5 minutes	006
Comments received (006); 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
Internal Standard Used?:	
No	006
ANALYSIS	
Sample injection volume (μl)	
1 -3 μl	006
GC injection mode:	
Splitless	006
GC COLUMN	
Column Type;	
Capillary	006
Column Make / Batch Number:	
Alltech, Catalog #13633, Serial # 5653, purchased in 1998, in continuous use	006
Column Packing:	
Chiral Phase: Chirasil-val	006
Column width (mm)	
0.25mm	006
Column length (mm)	
25m	006
Column Temperature ($^{\circ}\text{C}$):	
See below for program	006
Mobile phase / Carrier gas	
Helium	006
Mobile phase flow rate (ml/min):	
Flow variable with temperature; pressure 7.6psi	006

DETECTION	
Detector Type:	
Flame ionisation	006
Comments received (006); 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:	
<p>Comments received (006);</p> <p>Summary of the preparation sequence:</p> <ol style="list-style-type: none"> 1) Dissolution in stoichiometric amount of conc. HCl to bring final solution to 6N 2) Purge with N₂, seal hydrolysis tube, hydrolyse for 22 hours at 105 deg. 3) After hydrolysis, HCl solution is transferred to plastic centrifuge 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 to GC 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	001
Yes, within the last year	008
No	002, 003, 004, 005, 006, 009, 010, 011, 012, 013, 014, 015
If Yes, type of calibration:	
Calibration curve/std addition-single level	001
Calibrated by Agilent Technician	008
If Yes, what reference materials / standards are used?	
In-house std solution(s)	001
NB: Solution prepared from single powdered AA standards	
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	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
Norleucine	004, 005
No	006
Concentration of Internal std used (M):	
0.03 mM	001
0.01mM	002, 003, 008, 009, 010, 011, 012, 013, 014, 015
6.25 mM	004, 005
Source / supplier of internal standard:	
Sigma	001, 002, 003, 004, 005
Sigma Aldrich (Fluka)	008

D/L RATIO CALCULATION	
Do you routinely calculate concentrations?	
Yes	001, 009, 010, 011, 012, 013, 014, 015
No	002, 003, 004, 005, 006, 008
<p>Comments received;</p> <p>(001) Concentration of a single enantiomer in solution (milimol/L)= (enenatiomer area x Internal Standard concentration)/ Internal Standard area</p> <p>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)</p> <p>(006, 007): Only peak areas are reported under most circumstances but both are measured to check for reliability and peak distortion/overload.</p>	
D/L values are routinely calculated using:	
Peak heights	004, 005, 006
Peak areas	001, 002, 003, 006, 008
Concentrations based on peak areas	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, 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, 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, 008, 009, 010, 011, 012, 013, 014, 015
How do you use QC materials?	
Control charts	001, 002, 003, 004, 005
Visual inspection of chromatograms/data	008, 009, 010, 011, 012, 013, 014, 015
D/L comparison to lit	008
Comparison in ILC's with long term mean	006
MEASUREMENT UNCERTAINTY	
How do you determine Measurement Uncertainty (MU) of your data	
As the standard deviation	001, 002, 003, 004, 005, 006, 008, 009, 010, 011, 012, 013, 014, 015
If you do, how often do you determine the MU?	
Routinely per run	008
Approx once a month	002, 003, 004, 005,
When its needed	001, 009, 010, 011, 012, 013, 014, 015
As the SD of multiple chromatograms from each derivative.	006, 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 statistical 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;

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

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_{max}^2) to the sum of all the squared differences ($\sum D_i^2$) and comparing this against tabulated critical values. (ISO 5752-2: 1994)

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

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.; $y = (a + b + c \dots)$ then the combined standard uncertainty, $u_c(y)$ is given by;

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

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

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

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.

$$U = u_c \times k \quad \text{where } k = \text{the coverage factor, and} \\ u_c = \text{the combined uncertainty}$$

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;

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

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

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 $F = \frac{s_a^2}{s_b^2}$ or $= \frac{MS_{between}}{MS_{within}}$

(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. s_{all}^2 is derived from the homogeneity target standard deviation (either σ_p or σ_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 (\bar{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 trials.

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)

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

Relative Between Sample Standard Deviation ($RSD_L\%$), (expressed as a percentage)

The **between-sample standard deviation** divided by the (average) measurement result (x 100)

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

Relative Standard Uncertainty ($RSU\%$), (expressed as a percentage)

The **standard uncertainty** divided by the (average) measurement result (x 100)

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

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

The **standard deviation** divided by the (average) measurement result (x 100)

$$RSD\% \text{ or } CV\% = \left(\frac{S}{\bar{x}} \right) \times 100$$

Relative Repeatability standard deviation ($RSD_r\%$), (expressed as a percentage)

The **repeatability standard deviation** divided by the (average) measurement result (x 100)

$$RSD_r\% = (s_r/\bar{x}) \times 100$$

Relative Reproducibility standard deviation ($RSD_R\%$), expressed as a percentage

The **Reproducibility standard deviation** divided by the (average) measurement result (x 100)

$$RSD_R\% = (S_R/\bar{x}) \times 100$$

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;

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

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).

$$u(\hat{X}) = \hat{\sigma} / \sqrt{m} \quad \text{where } \hat{\sigma} = \text{the assigned value std dev}$$

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;

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

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).

$$u(\bar{x}) = \frac{s_{\bar{x}}}{\sqrt{n}} \quad \text{where } s_{\bar{x}} = \text{the std dev of replicate values}$$

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 trial 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
$$t = \frac{(x - \mu)}{s/\sqrt{n}}$$

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.

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

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 $|z| \leq 2$.

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

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)

Appendix 4: References

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