LABORATORY NAME:

LABORATORY NUMBER:

Amino Acid Racemization Proficiency Study

Report I: OPERCULA

June 2012

Acknowledgements.

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1 INTRODUCTION

1.1 Amino Acid Racemization

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

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

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

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

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

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

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

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

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

1.2 Proficiency Testing

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

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

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

[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
Opercula

2.1 Preparation

The calcitic opercula test material was prepared from a 2 g bulk of individual *Bithynia tentaculata* opercula, removed from previously collected sediment (28 July 2005) taken from a UK Quaternary site located in Funtham's Lane, approximately 5km east of Peterborough, in Cambridgeshire, United Kingdom (Langford et al., 2007, Penkman et al., 2007, Penkman et al., 2008).

After sieving and extracting sufficient individual opercula, the material was cleaned. Large pieces of extraneous matter that could be removed from individual opercula, were removed and the bulk material was then repeatedly washed in ultrapure water using a sonicator until the water remained clear. The cleaned opercula were then lightly covered and left to air dry for 48 hours. Following this, the bulk material was ground using a sterile pestle and mortar and sieved, to ≤ 250 µm and was then tumble-blended overnight on a roller mixer. The powdered opercula were then bleached with intermittent shaking, for 48 hours using 50µl of 12% NaOCl per mg of powder. The bleach was removed and the powder washed with ultrapure water up to six times using a vortex mixer followed by centrifugation to pellet the solids in between washes. A final wash with methanol to remove any remaining water was carried out before the material was again lightly covered and left to air dry.

Individual 20mg sub-samples of the cleaned, bleached and dried opercula powder were weighed into sterile glass vials, labelled and 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

Opercula 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;

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

Where; *C* is the Cochran's statistic,

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

D_i is the difference between each pair of duplicates.

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

b) Evaluation of Analytical Variance

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

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

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

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

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

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

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

c) Evaluation of Sampling Variance.

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

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

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

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

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

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

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

3.2 Evaluation of Opercula 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 the total hydrolysed amino acids. The twenty individual sub-samples where then randomized and analysed as a single batch under repeatability conditions using reverse-phase HPLC.

During the analytical run, instrumental problems occurred. Investigations were carried out to stabilize the pressure which extended the total run time by a number of days, and resulted in the column finally being changed for the last four data points. The D/L results for each amino acid were plotted in run order to identify trends or problems with the data and are shown in Figure 3.1.

Data points obtained using the new column have been coloured yellow. The D/L results and statistical evaluation are given in Table 3.1. Values identified as outliers were removed as a pair from the evaluation. These have been coloured red in the tables. Figure 5.2 shows the paired D/L values for each amino acid. Outliers that were removed from the statistical evaluation are shown as empty symbols on the charts.

For all amino acids, results for the last four data points (4a, 9a, 7b and 2a) run on the new column have been considered anomalous as they have not been analysed under true repeatability conditions. Results for these data points show discrepancies with the earlier results for several amino acids. Whilst this may be due to calibration issues, for glutamic acid, valine and possibly serine, these observations are not present. This suggests that any offset could also be due to instability of the sample extracts whilst waiting the 3.5 days from the point of instrument failure and investigation to restarting the analysis at sub-sample 4a.

From the time series plots it can also be seen that sub-sample 7a gave anomalous results for all amino acids. This may have been due to genuine inhomogeneity in the test materials or sample preparation problems such as incomplete hydrolysis or contamination etc. However, sub-sample 7a has already been removed for evaluation purposes as it is paired with one of the last four anomalous data points. For several amino acids it almost appears as if the analysis did not fully recover following sample 7a and prior to replacing the column. It is possible that these results could have been affected by instrument instability during this phase before completely failing.

Due to the problems discussed above, the number of remaining pairs of data was reduced to six, which is below the minimum recommended sample size. Ideally the run should have been repeated but time did not permit this. Looking at the results for the first eleven individual sub-samples, there is no indication of inhomogeneity. The only variation observed being indicative of the precision of the measurement procedure and the prior expectations for the different amino acids from previous study. Having taken every precaution to ensure test materials were prepared homogeneously it was considered acceptable to continue the assessment on the remaining data. Critical values were extrapolated to accommodate a smaller data set.

A further Cochran's outlier was identified for aspartic acid (sample 8), and borderline outliers were observed for arginine and alanine. In both cases sub-sample 3b can be seen to lie outside the general distribution of results in Figure 3.1. Whilst it would have been statistically acceptable to retain sample 3, especially considering the low number of results remaining for evaluation, removal of this pair in both cases made a noticeable difference and resulted in a target value for standard deviation that better reflected the level of agreement for the remaining data.

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 Opercula Test Material

sample id	analyte									
	Asx D/L		Glx D/L		Ser D/L		Arg D/L		Ala D/L	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
1	0.582	0.584	0.168	0.167	0.667	0.665	0.726	0.726	0.260	0.264
2	0.570	0.584	0.169	0.173	0.659	0.668	0.911	0.794	0.287	0.257
3	0.584	0.573	0.168	0.167	0.662	0.671	0.755	1.295 C	0.259	0.286 C
4	0.570	0.585	0.168	0.168	0.658	0.670	1.245	0.649	0.295	0.255
5	0.585	0.581	0.168	0.167	0.674	0.654	0.879	0.686	0.267	0.262
6	0.579	0.580	0.167	0.168	0.666	0.675	0.850	0.863	0.247	0.254
7	0.522	0.571	0.130	0.169	0.315	0.640	0.546	0.765	0.225	0.293
8	0.554	0.580 C	0.167	0.168	0.655	0.666	0.744	0.895	0.250	0.252
9	0.570	0.579	0.170	0.167	0.656	0.652	0.777	0.755	0.290	0.259
10	0.580	0.578	0.165	0.165	0.640	0.649	0.850	0.695	0.261	0.250
mean, N	0.581	10	0.167	12	0.662	12	0.791	10	0.257	10
origin of target sd (σ_h)	perception		perception		perception		perception		perception	
abs. target sd (σ_h) & as RSD%	0.0077	1.33	0.0012	0.7	0.0163	2.46	0.1836	23.2	0.0096	3.75
s_{an}	0.0038		0.0006		0.0081		0.0917		0.0048	
s_{an} / σ_h	0.4981		0.4997		0.4981		0.4992		0.4986	
s_{an} / σ_h <0.5?	yes		yes		yes		yes		yes	
s_{sam}^2	0.00E+00		8.01E-07		5.41E-05		0.00E+00		2.83E-05	
σ_{all}^2	5.37E-06		1.23E-07		2.39E-05		3.03E-03		8.34E-06	
critical	3.97E-05		8.09E-07		1.56E-04		2.26E-02		6.19E-05	
s_{sam}^2<critical?	ACCEPT		ACCEPT		ACCEPT		ACCEPT		ACCEPT	

Table 3.1: Homogeneity D/L Values for Opercula 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	0.131	0.135	0.303	0.299	0.170	0.171	0.248	0.250
2	0.131	0.136	0.307	0.299	0.118	0.168	0.301	0.247
3	0.136	0.133	0.296	0.294	0.170	0.165	0.248	0.233
4	0.130	0.135	0.299	0.296	0.186	0.179	0.284	0.261
5	0.135	0.134	0.301	0.288	0.171	0.164	0.248	0.242
6	0.130	0.135	0.294	0.293	0.155	0.166	0.241	0.245
7	0.106	0.127	0.231	0.310	0.131	0.136	0.195	0.299
8	0.134	0.131	0.297	0.305	0.170	0.171	0.248	0.246
9	0.132	0.129	0.315	0.297	0.137	0.167	0.306	0.242
10	0.134	0.133	0.288	0.287	0.163	0.169	0.241	0.247
mean, N	0.133	12	0.296	12	0.167	12	0.245	12
origin of target sd (σ_h)	perception		perception		perception		perception	
abs. target sd (σ_h) & as RSD%	0.0046	3.45	0.0093	3.16	0.0085	5.06	0.0103	4.2
s_{an}	0.0023		0.0047		0.0042		0.0051	
s_{an} / σ_h	0.4990		0.4997		0.4995		0.4966	
s_{an} / σ_h <0.5?	yes		yes		yes		yes	
s_{sam}^2	0.00E+00		1.46E-05		4.05E-06		0.00E+00	
σ_{all}^2	1.91E-06		7.85E-06		6.44E-06		9.51E-06	
critical	1.25E-05		5.16E-05		4.23E-05		6.20E-05	
s_{sam}^2<critical?	ACCEPT		ACCEPT		ACCEPT		ACCEPT	

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

(Note; yellow data points represent D/L values derived using the replacement HPLC column)

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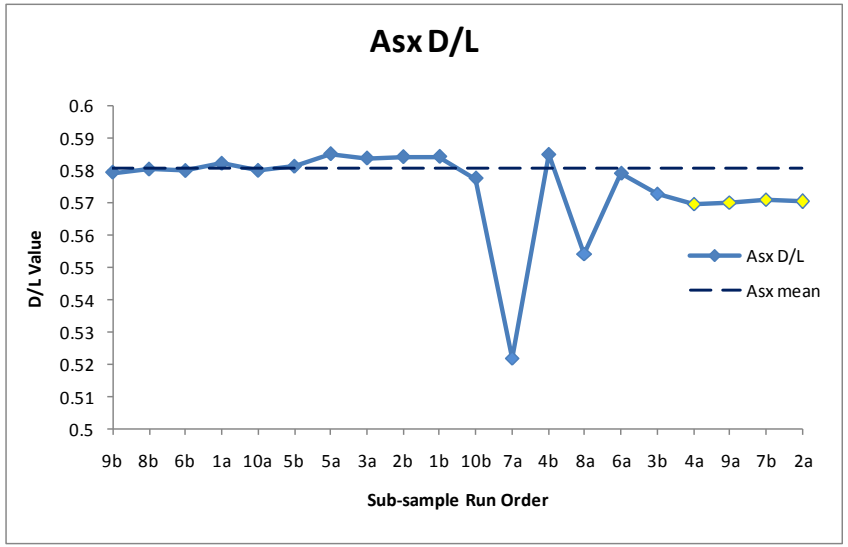

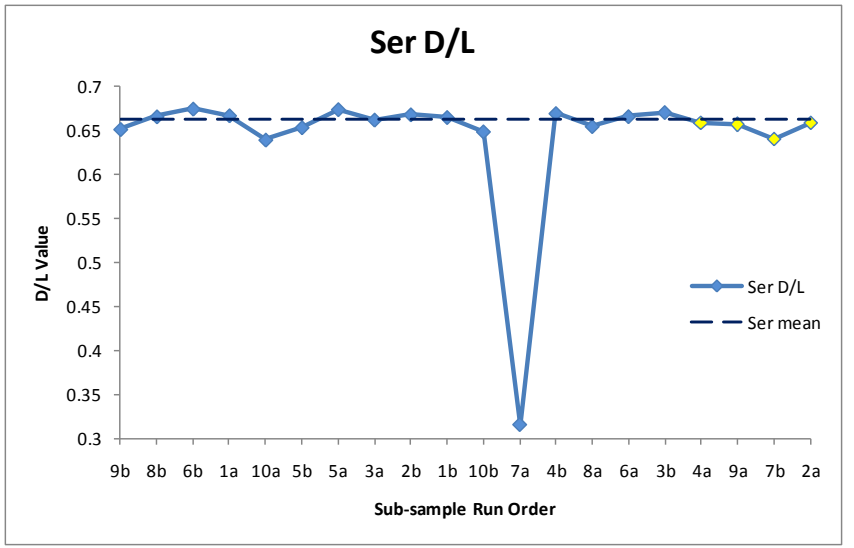

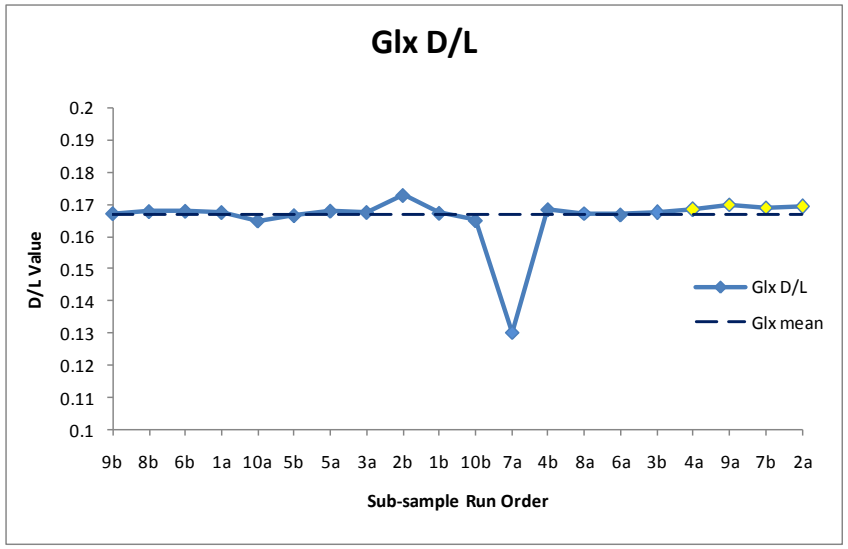

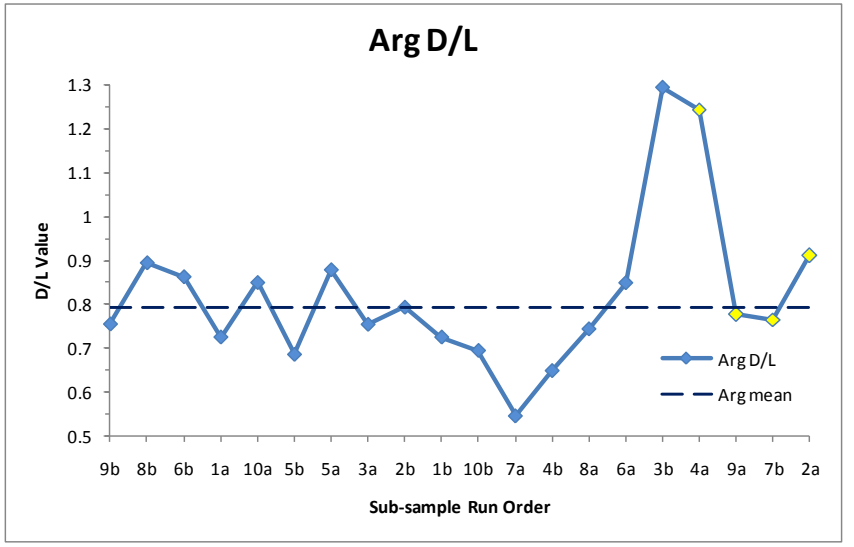

Figure 3.1: Homogeneity Amino Acid D/L Values in Analytical Sequence Order (continued)

(Note; yellow data points represent D/L values derived using the replacement HPLC column)

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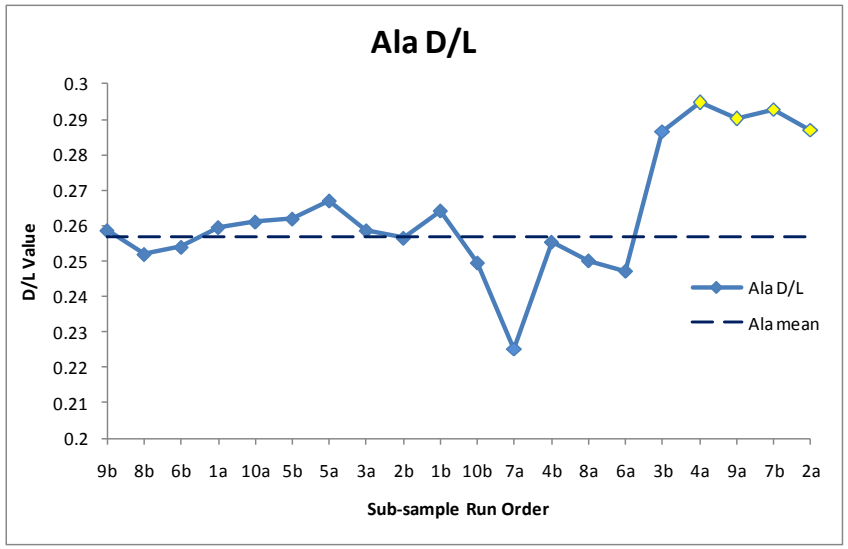
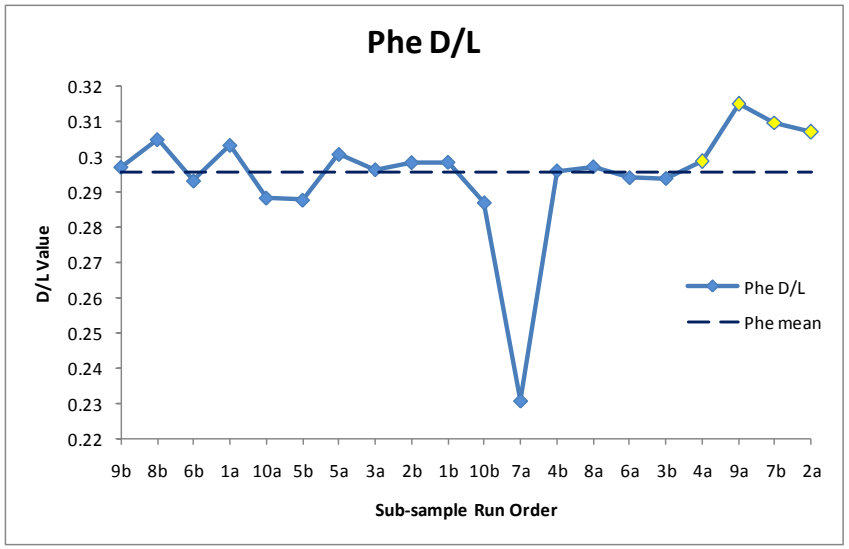
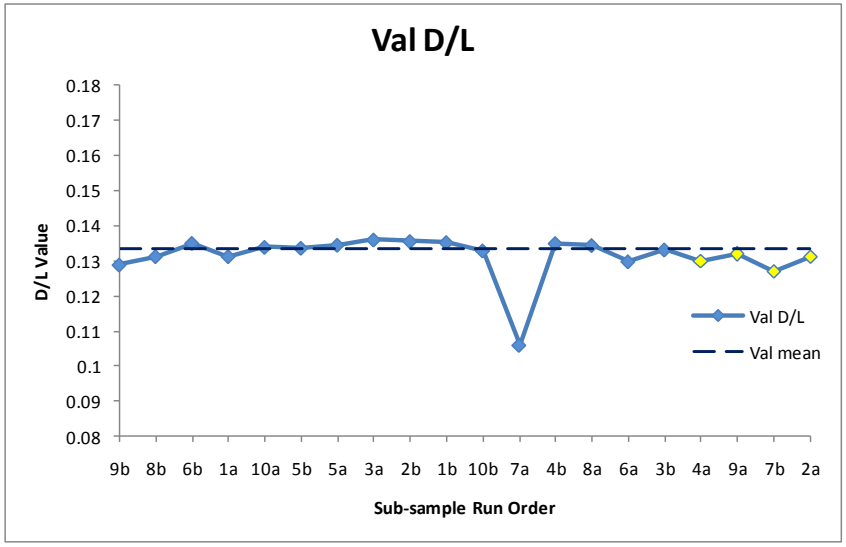






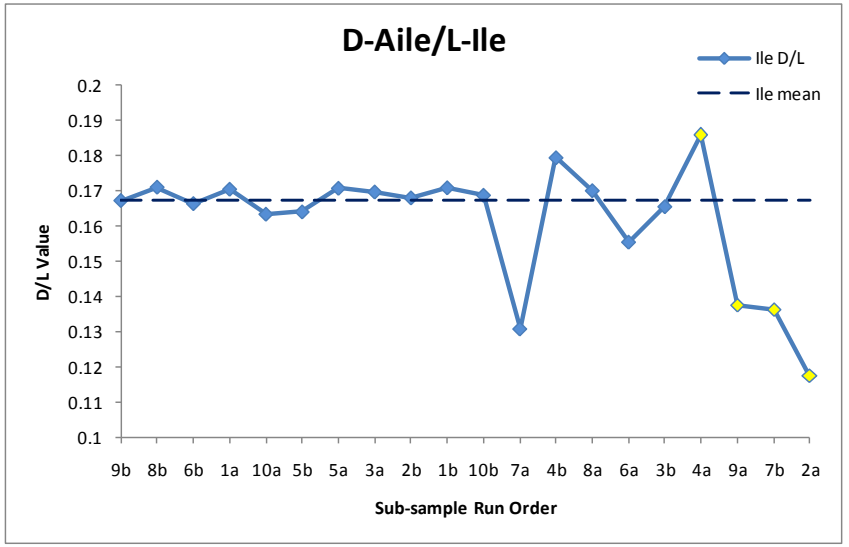


Figure 3.1: Homogeneity Amino Acid D/L Values in Analytical Sequence Order; (continued)

(Note; yellow data points represent D/L values derived using the replacement HPLC column)

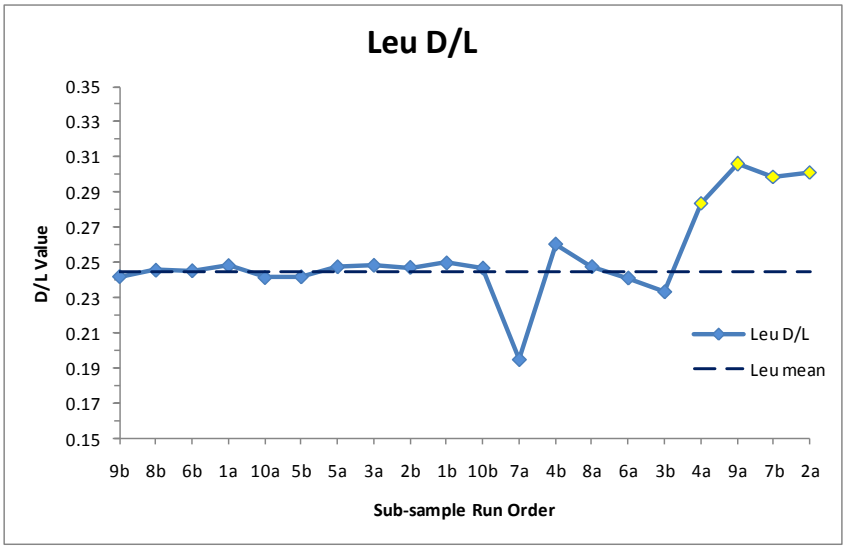


Figure 3.2: Homogeneity Amino Acid D/L Values; Paired Sub-samples showing Outliers.

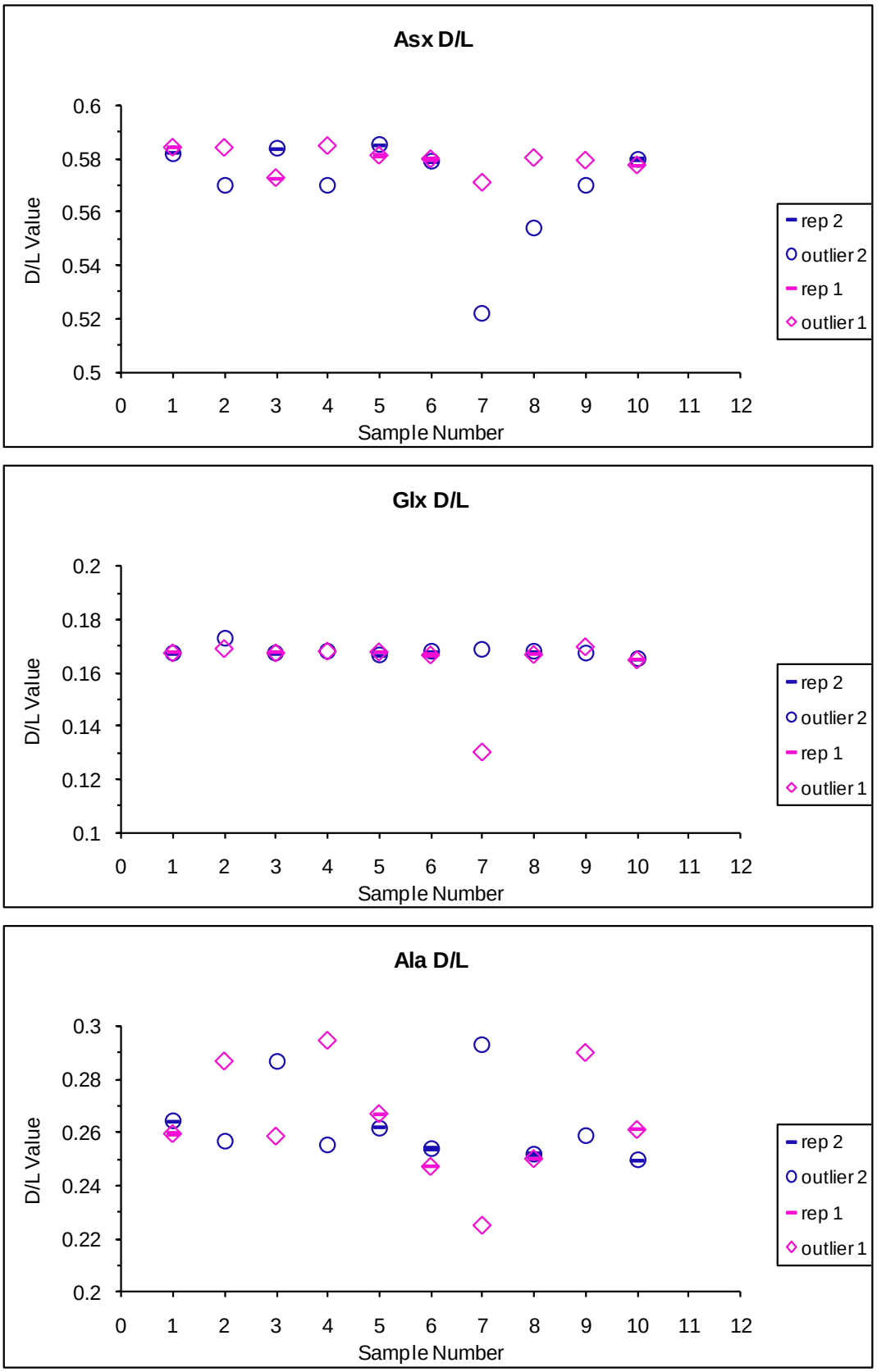


Figure 3.2: Homogeneity Amino Acid D/L Values; Paired Sub-samples showing Outliers.

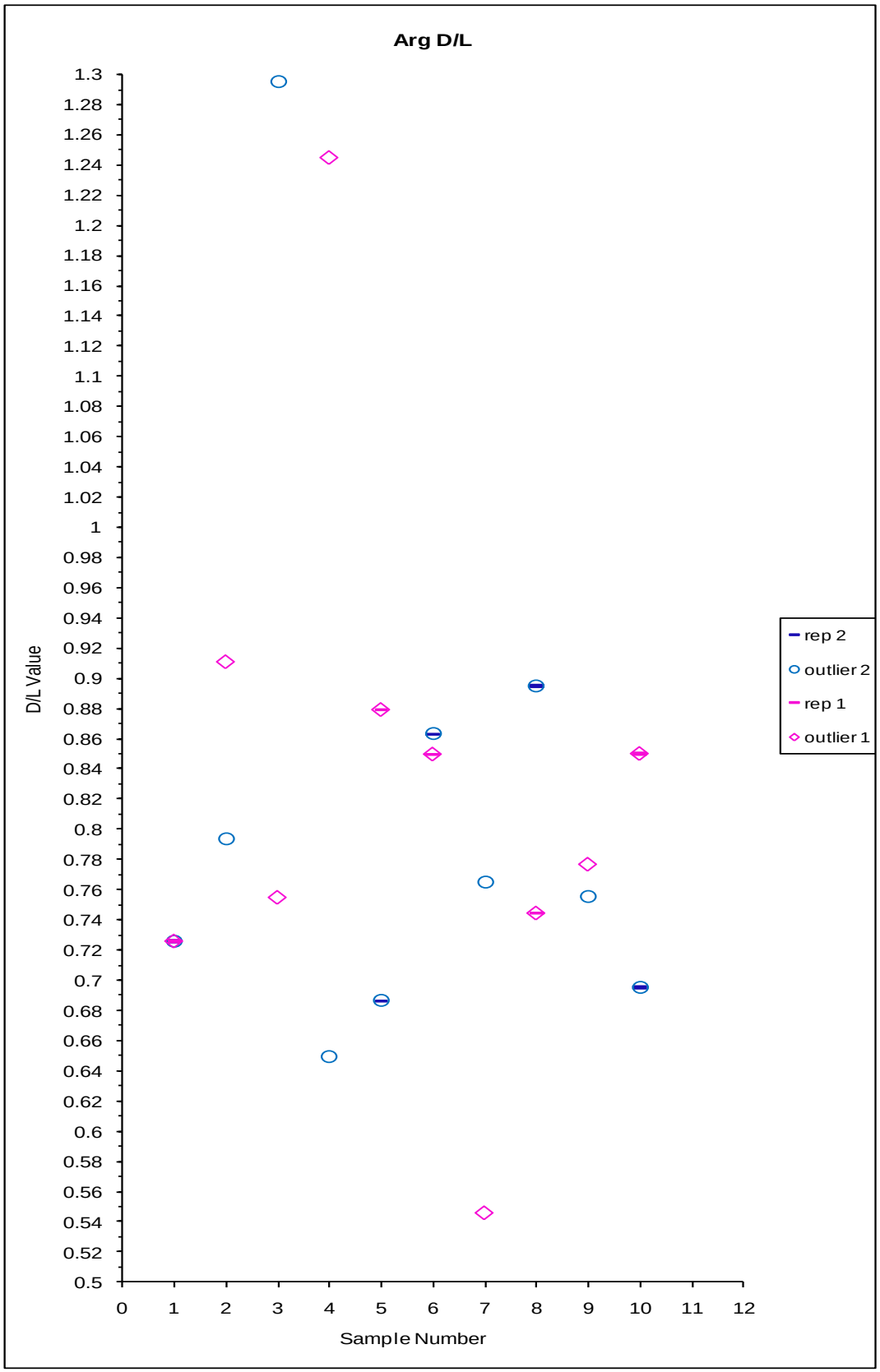


Figure 3.2: Homogeneity Amino Acid D/L Values; Paired Sub-samples showing Outliers.

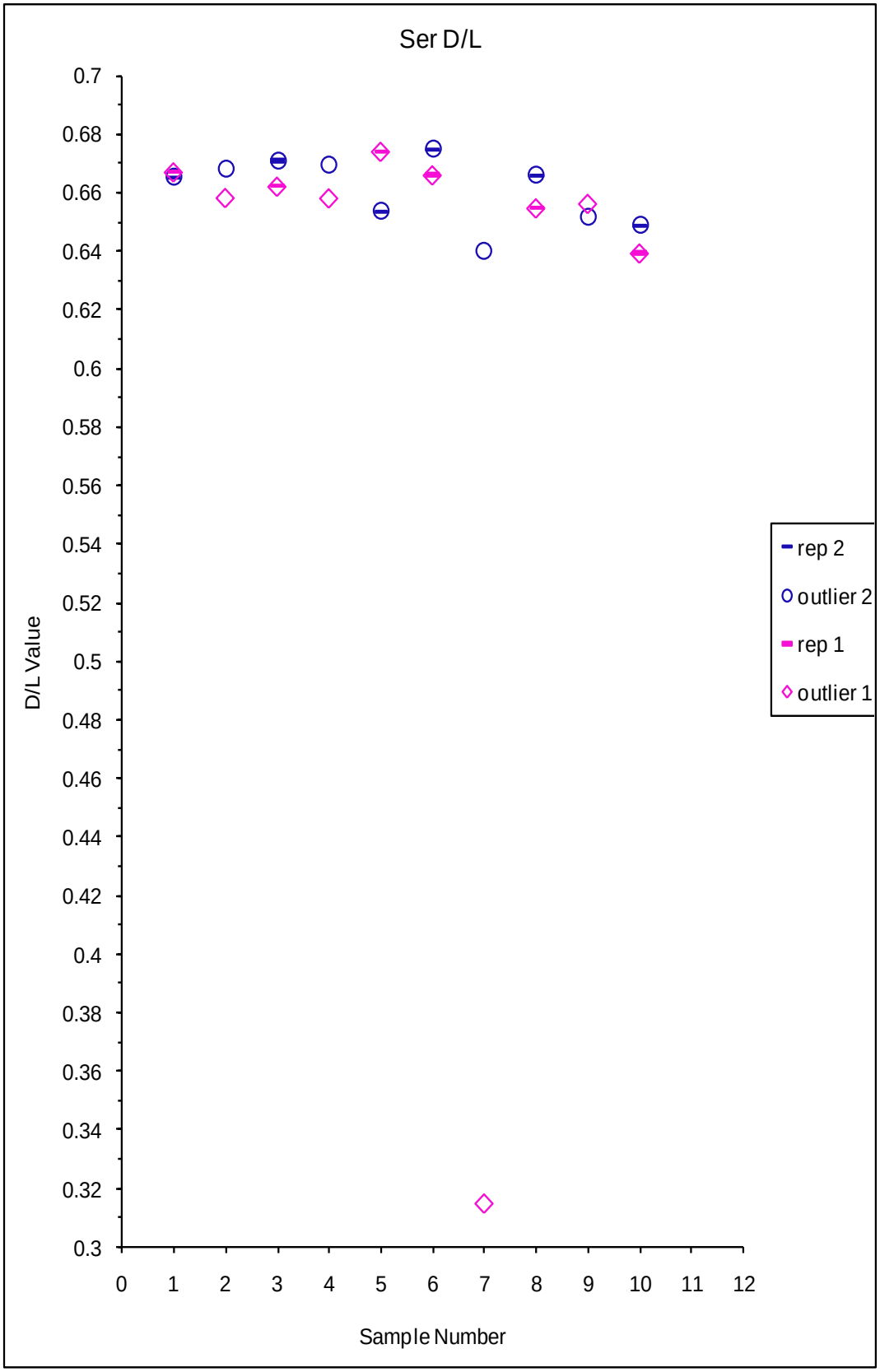


Figure 3.2: Homogeneity Amino Acid D/L Values; Paired Sub-samples showing Outliers.

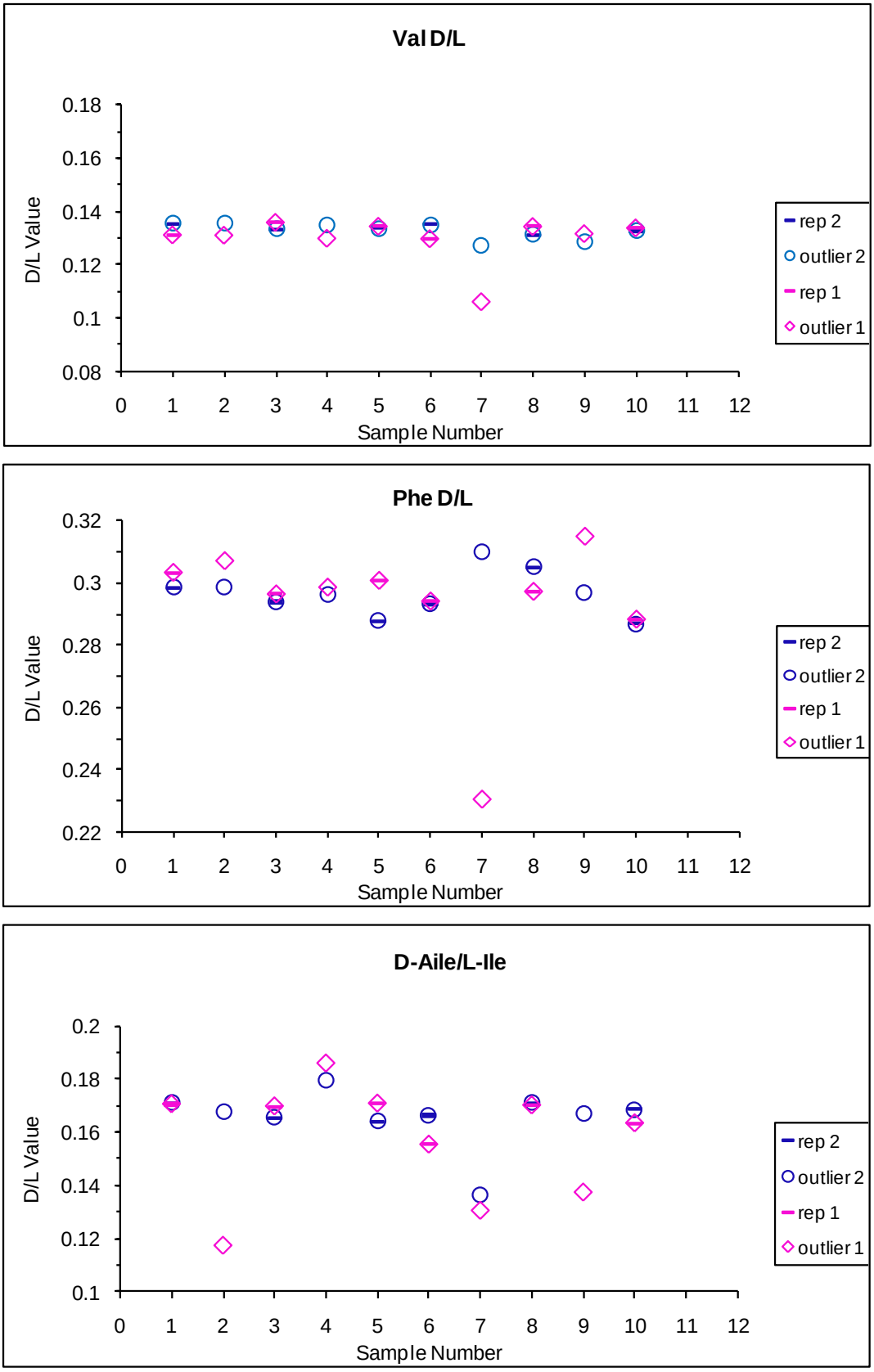
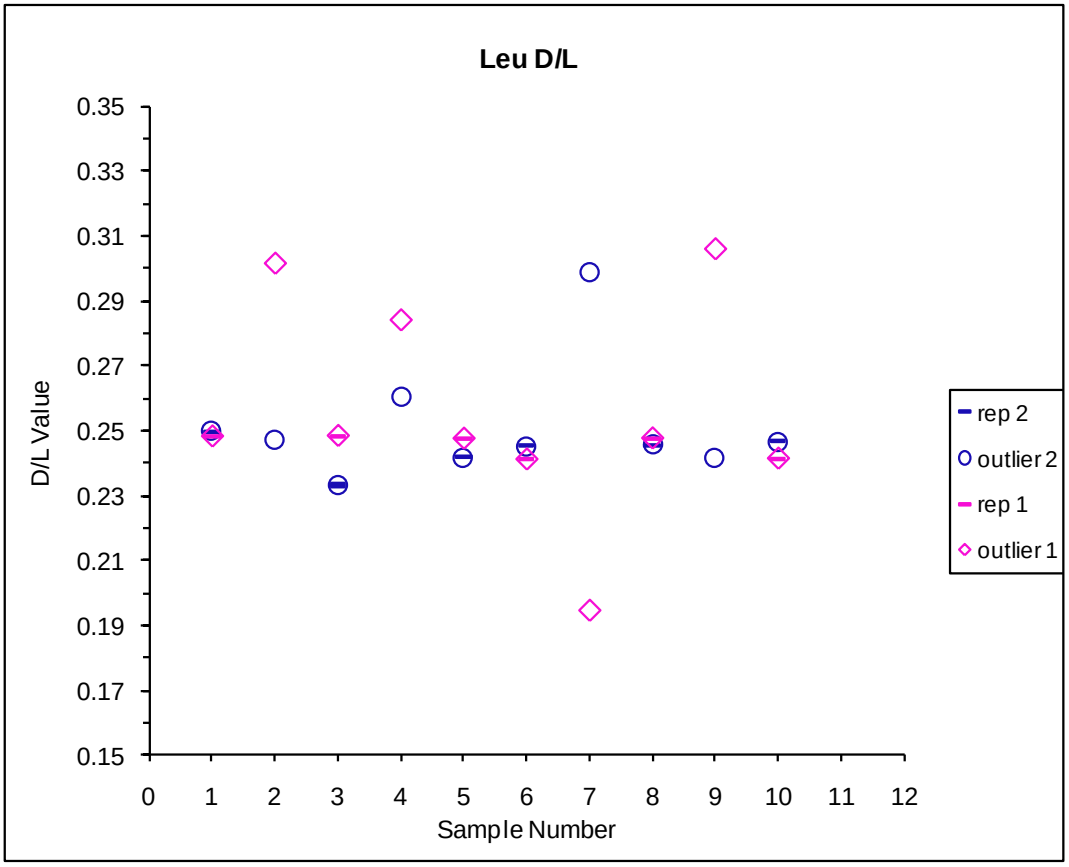


Figure 3.2: Homogeneity Amino Acid D/L Values; Paired Sub-samples showing Outliers.



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 trail data. On this occasion it is the intention to observe the behaviour of all submitted results rather than to define best practice. It should be noted that these values have not been used in the later performance evaluation but are given for information and indicative purposes only. Further details on the calculations of S_R, S_L and S_r can be found in (ISO 5725, 1994, ISO 21748, 2010). Precision estimates are calculated using ANOVA, thus;

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

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

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

Table 4.1: Precision Estimates derived from Participants' submitted results

amino acid	no of sets of results (m)	total no of replicates (N)	mean	S_r	$RSD_r\%$	S_L	$RSD_L\%$	S_R	$RSD_R\%$
Asx D/L-all[a]	13	31	0.569	0.0030	0.53	0.0242	4.25	0.0243	4.28
Asx D/L-rpHPLC	11	29	0.564	0.0030	0.54	0.0134	2.37	0.0137	2.43
Glx D/L-all[a]	13	31	0.159	0.0008	0.51	0.0130	8.16	0.0130	8.18
Glx D/L-rpHPLC	11	29	0.157	0.0008	0.52	0.0098	6.21	0.0098	6.23
Ser D/L-rpHPLC	11	29	0.656	0.0073	1.12	0.0090	1.37	0.0116	1.77
Arg D/L-rpHPLC	9	17	0.776	0.1515	19.53	0.1551	19.99	0.2168	27.95
Ala D/L-all[a]	13	31	0.267	0.0054	2.01	0.0116	4.36	0.0128	4.80
Ala D/L-rpHPLC	11	29	0.268	0.0054	2.01	0.0115	4.28	0.0127	4.72
Val D/L-all[a]	13	31	0.135	0.0059	4.35	0.0085	6.31	0.0103	7.67
Val D/L-rpHPLC	11	29	0.135	0.0059	4.33	0.0073	5.41	0.0094	6.93
Phe D/L-all[a]	13	31	0.305	0.0158	5.20	0.0087	2.86	0.0181	5.93
Phe D/L-rpHPLC	11	29	0.306	0.0158	5.18	0.0086	2.82	0.0180	5.90
D-Aile/L-Ile-all[b]	15	35	0.185	0.0252	13.62	0.0587	31.77	0.0639	34.57
D-Aile/L-Ile -rpHPLC	11	29	0.194	0.0265	13.65	0.0616	31.70	0.0671	34.51
D-Aile/L-Ile -HPLC-IE	2	4	0.137	0.0034	2.45	0.0021	1.55	0.0040	2.90
D-Aile/L-Ile -GC	Not determined								
Leu D/L-all[a]	10	26	0.283	0.0234	8.28	0.0396	13.99	0.0460	16.26
Leu D/L-rpHPLC	8	24	0.289	0.0234	8.11	0.0350	12.14	0.0421	14.60
Tyr D/L-rpHPLC	5	10	0.273	0.0061	2.24	0.0135	4.94	0.0148	5.42

[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;

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

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

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

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

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

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

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

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

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

4.2.2 Setting the correct coverage factor for Expanded Uncertainty determination.

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

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

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

$$\bar{x} - \left[t_{(2,o.o5,df)} \times \frac{\sigma}{\sqrt{n}}\right] \quad to \quad \bar{x} + \left[t_{(2,o.o5,df)} \times \frac{\sigma}{\sqrt{n}}\right]$$

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

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

The differences observed in expanded uncertainties between different amino acids for a single laboratory highlights the ease or difficulty of analysis and instrument repeatability. A comparison of

expanded uncertainties across all laboratories for any individual amino acid also demonstrates the effect of different methods or even using different numbers of replicates for the same method.

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

4.3 t-Distribution vs Normal Distribution

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

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

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

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

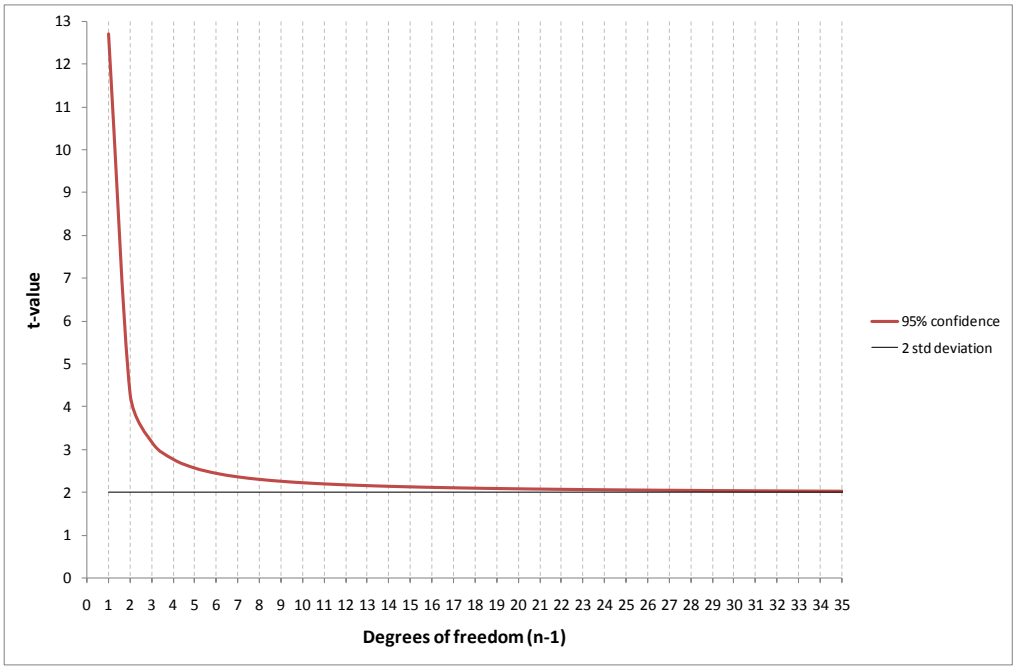


Table 4.2: Summary Statistics for L and D **Aspartic Acid / Asparagine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Asx peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
001	RP	21681	21911	22843	23297	24257	24549	25623	25904	27040	27893	24500	10	2110.8	8.62	667.5	2.72	5.45	2.262	6.16
002	RP	3167	3216									3192	2	34.7	1.09	24.5	0.77	1.54	12.710	9.77
003	RP	3291										3291	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	29046	28737									28892	2	218.5	0.76	154.5	0.53	1.07	12.710	6.80
009	RP	13699	13791									13745	2	65.5	0.48	46.3	0.34	0.67	12.710	4.28
010	RP	8102	8654									8378	2	390.7	4.66	276.2	3.30	6.59	12.710	41.91
011	RP	6586	6600									6593	2	9.6	0.15	6.8	0.10	0.21	12.710	1.31
012	RP	12134	12273									12203	2	98.4	0.81	69.6	0.57	1.14	12.710	7.25
013	RP	30202	30018									30110	2	130.2	0.43	92.0	0.31	0.61	12.710	3.88
014	RP	12373	12392									12382	2	13.9	0.11	9.8	0.08	0.16	12.710	1.01
015	RP	12589	12221									12405	2	260.0	2.10	183.8	1.48	2.96	12.710	18.84
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% ($k=t_{crit}$)**
001	RP	11890	12030	12555	12752	13326	13500	14058	14211	14868	15335	13452	10	1167.6	8.68	369.2	2.74	5.49	2.262	6.21
002	RP	1758	1766									1762	2	5.8	0.33	4.1	0.23	0.46	12.710	2.94
003	RP	1879										1879	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	16714	16566									16640	2	105.1	0.63	74.3	0.45	0.89	12.710	5.68
009	RP	7905	7941									7923	2	25.0	0.32	17.7	0.22	0.45	12.710	2.83
010	RP	4623	4945									4784	2	227.6	4.76	161.0	3.36	6.73	12.710	42.77
011	RP	3869	3781									3825	2	62.4	1.63	44.1	1.15	2.31	12.710	14.65
012	RP	7008	7085									7047	2	53.8	0.76	38.0	0.54	1.08	12.710	6.86
013	RP	17284	17192									17238	2	64.9	0.38	45.9	0.27	0.53	12.710	3.38
014	RP	7091	7049									7070	2	29.8	0.42	21.0	0.30	0.60	12.710	3.78
015	RP	7220	6926									7073	2	208.2	2.94	147.2	2.08	4.16	12.710	26.45

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Asx Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
001	RP	1273	1264	1235	1259	1266	1260	1260	1271	1256	1274	1262	10	11.3	0.89	3.6	0.28	0.57	2.262	0.64
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	1524	1517									1521	2	5.3	0.35	3.8	0.25	0.50	12.710	3.16
009	RP	1546	1652									1599	2	74.9	4.69	53.0	3.31	6.63	12.710	42.12
010	RP	1658	1630									1644	2	19.9	1.21	14.1	0.86	1.71	12.710	10.90
011	RP	1310	1412									1361	2	72.0	5.29	50.9	3.74	7.48	12.710	47.53
012	RP	1435	1591									1513	2	110.0	7.27	77.8	5.14	10.28	12.710	65.31
013	RP	980	980									980	2	0.1	0.01	0.1	0.01	0.02	12.710	0.10
014	RP	1206	1604									1405	2	281.8	20.06	199.3	14.18	28.36	12.710	180.25
015	RP	1312	1533									1422	2	155.9	10.96	110.2	7.75	15.50	12.710	98.50
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% ($k=t_{crit}$)**
001	RP	698	694	679	689	696	693	692	697	691	700	693	10	6.1	0.88	1.9	0.28	0.56	2.262	0.63
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	877	874									876	2	2.0	0.23	1.4	0.16	0.32	12.710	2.04
009	RP	892	951									921	2	41.7	4.53	29.5	3.20	6.40	12.710	40.67
010	RP	946	931									939	2	10.5	1.12	7.4	0.79	1.58	12.710	10.04
011	RP	770	809									789	2	27.7	3.51	19.6	2.48	4.97	12.710	31.58
012	RP	829	918									874	2	63.1	7.22	44.6	5.11	10.22	12.710	64.92
013	RP	561	561									561	2	0.2	0.04	0.2	0.03	0.06	12.710	0.40
014	RP	691	913									802	2	156.6	19.53	110.8	13.81	27.62	12.710	175.55
015	RP	753	869									811	2	82.0	10.12	58.0	7.15	14.31	12.710	90.93

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Asx		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
001	RP	0.548	0.549	0.550	0.547	0.549	0.550	0.549	0.549	0.550	0.550	0.549	10	0.0008	0.15	0.0003	0.05	0.09	2.262	0.11
002	RP	0.555	0.549									0.552	2	0.0042	0.76	0.0030	0.54	1.08	12.710	6.83
003	RP	0.571										0.571	1							
004	IE																			
005	IE																			
006[1]	GC_A	0.650										0.650	1							
007[1]	GC_A	0.631										0.631	5	0.0450	7.13	0.0201	3.19	6.38	2.777	8.86
008	RP	0.575	0.576									0.576	2	0.0007	0.12	0.0005	0.09	0.17	12.710	1.10
009	RP	0.577	0.576									0.576	2	0.0009	0.16	0.0007	0.11	0.23	12.710	1.45
010	RP	0.571	0.571									0.571	2	0.0005	0.10	0.0004	0.07	0.14	12.710	0.86
011	RP	0.587	0.573									0.580	2	0.0103	1.78	0.0073	1.26	2.51	12.710	15.97
012	RP	0.578	0.577									0.577	2	0.0003	0.04	0.0002	0.03	0.06	12.710	0.39
013	RP	0.572	0.573									0.573	2	0.0003	0.06	0.0002	0.04	0.08	12.710	0.50
014	RP	0.573	0.569									0.571	2	0.0030	0.53	0.0022	0.38	0.75	12.710	4.79
015	RP	0.574	0.567									0.570	2	0.0048	0.85	0.0034	0.60	1.20	12.710	7.62

[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**

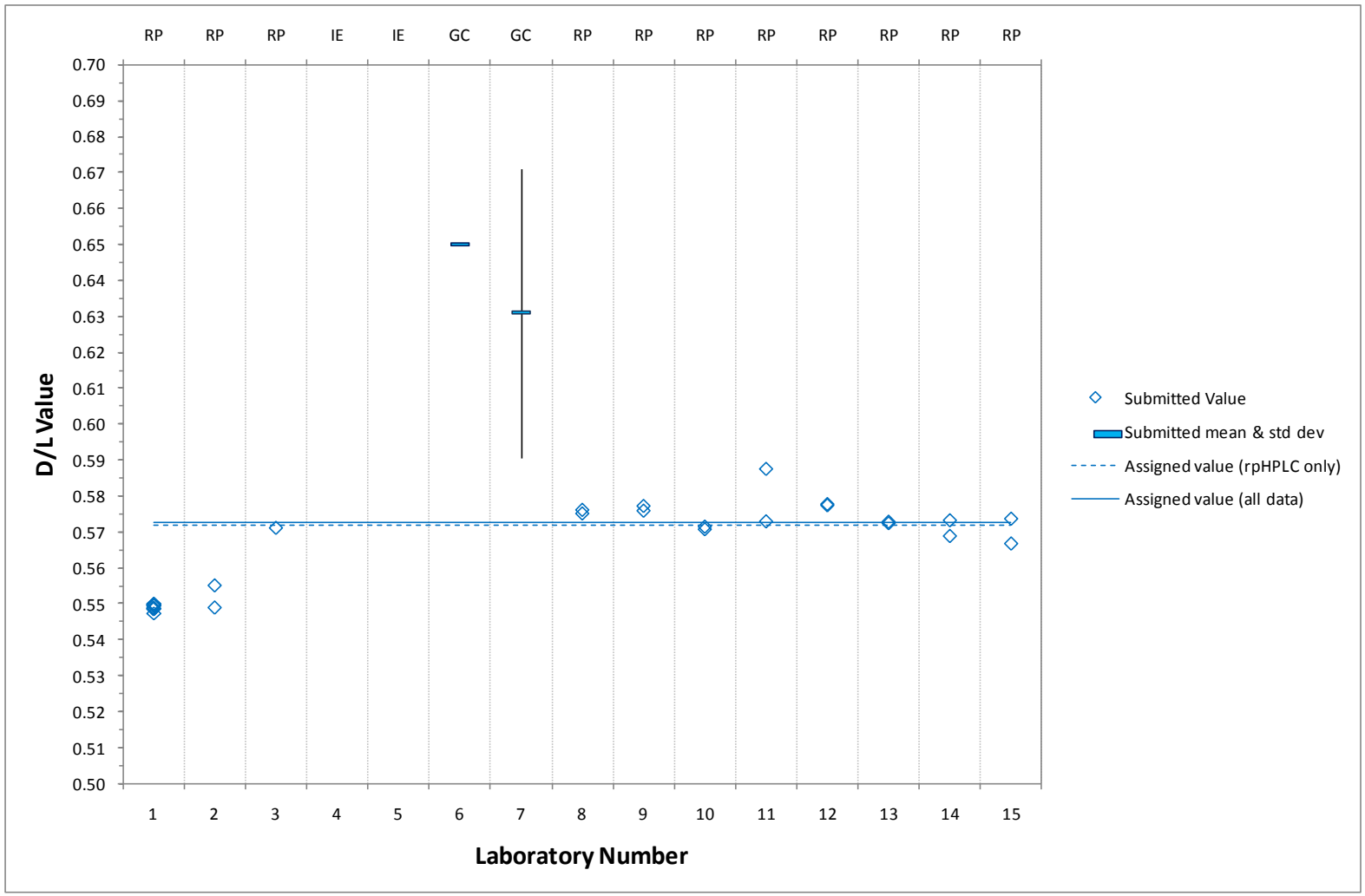


Figure 4.3: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Aspartic Acid / Asparagine** (value of n displayed).

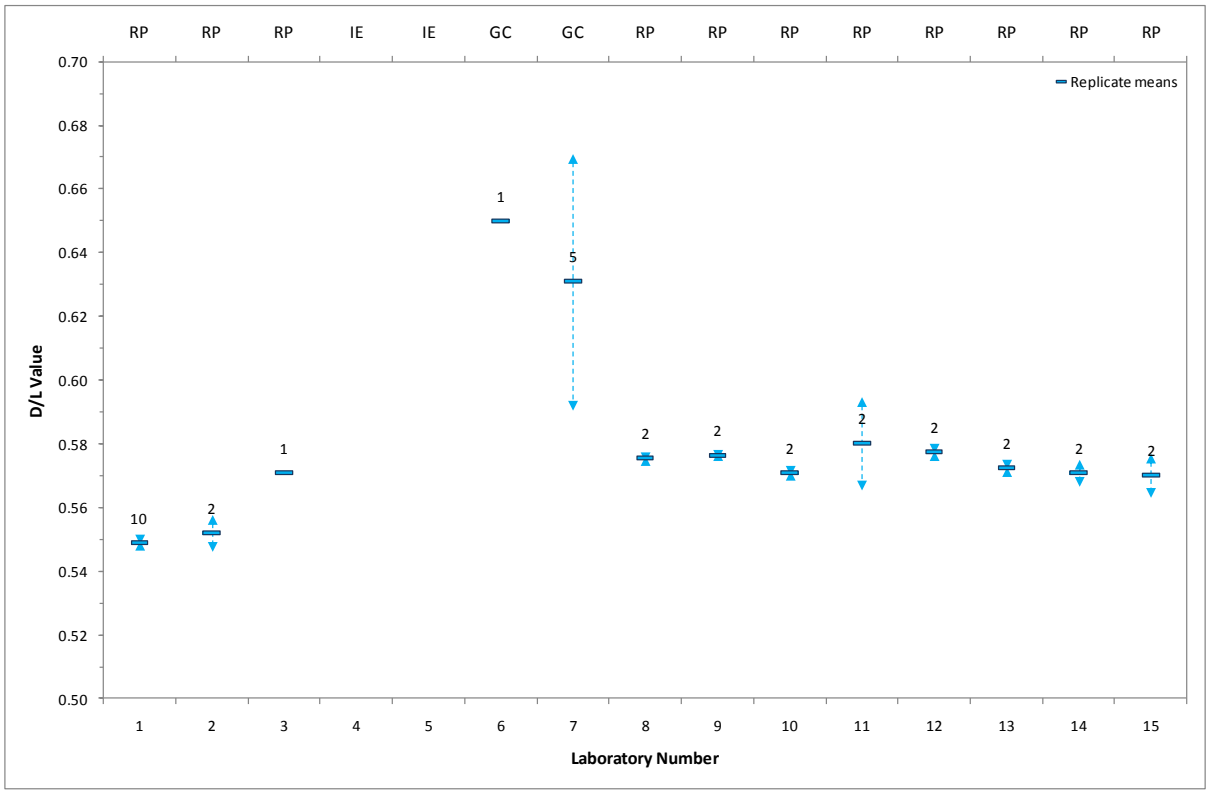


Figure 4.4: Experimental Expanded Uncertainty **(k=$t_{(0.05,df)}$)** of the Mean D/L value for **Aspartic Acid / Asparagine** (value of n displayed).

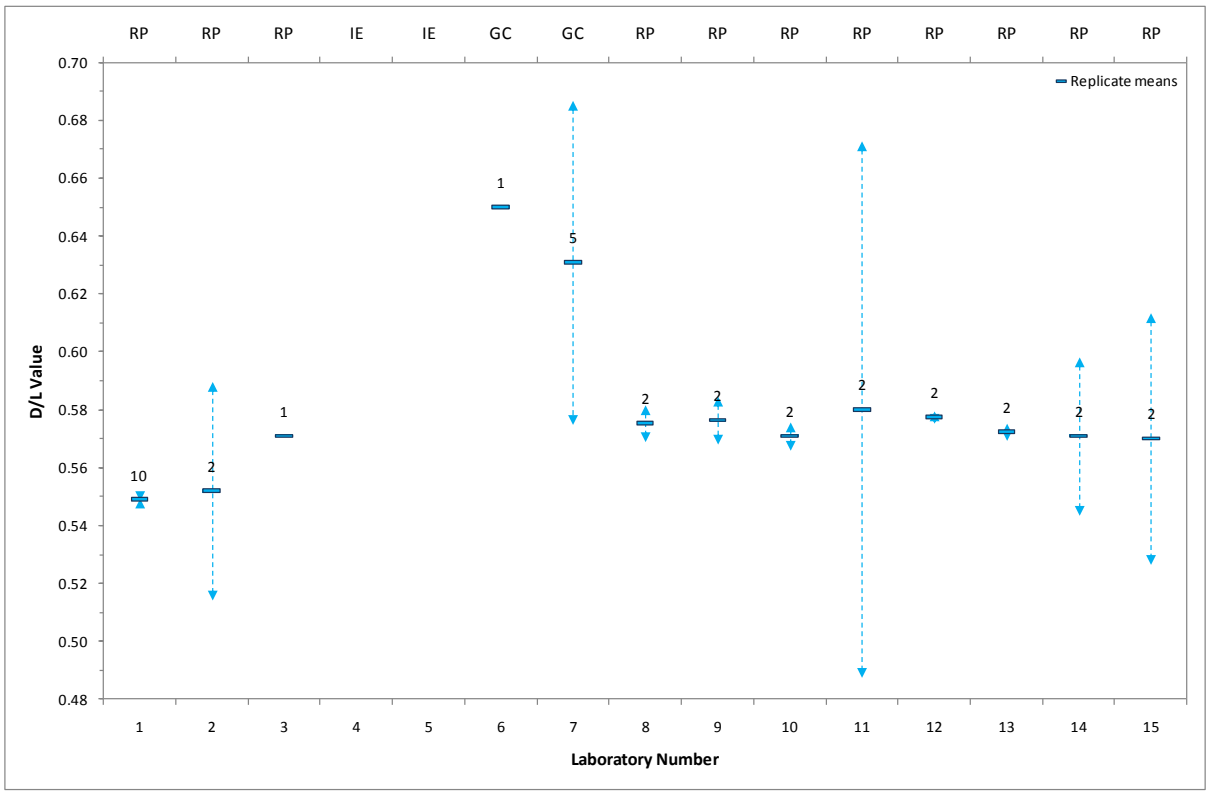


Table 4.5: Summary Statistics for L and D **Glutamic Acid / Glutamine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Glx peak area		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% ($k=t_{crit}$)
001	RP	18568	18834	19993	20309	20852	20977	21899	22095	22910	23473	20991	10	1629.2	7.76	515.2	2.45	4.91	2.262	5.55
002	RP	2485	2517									2501	2	22.6	0.90	16.0	0.64	1.28	12.710	8.13
003	RP	2636										2636	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	24048	23772									23910	2	195.3	0.82	138.1	0.58	1.15	12.710	7.34
009	RP	11024	10948									10986	2	53.4	0.49	37.7	0.34	0.69	12.710	4.37
010	RP	6490	6736									6613	2	174.2	2.63	123.1	1.86	3.72	12.710	23.67
011	RP	5251	5270									5260	2	13.0	0.25	9.2	0.17	0.35	12.710	2.22
012	RP	9525	9588									9556	2	44.5	0.47	31.5	0.33	0.66	12.710	4.19
013	RP	25859	25701									25780	2	111.9	0.43	79.1	0.31	0.61	12.710	3.90
014	RP	9965	9763									9864	2	142.8	1.45	101.0	1.02	2.05	12.710	13.01
015	RP	10440	10261									10350	2	127.0	1.23	89.8	0.87	1.73	12.710	11.02
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% ($k=t_{crit}$)
001	RP	2757	2790	3008	3062	3127	3146	3270	3294	3433	3511	3140	10	248.9	7.93	78.7	2.51	5.01	2.262	5.67
002	RP	352	349									351	2	2.1	0.59	1.5	0.42	0.83	12.710	5.29
003	RP	380										380	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	3915	3878									3896	2	26.4	0.68	18.6	0.48	0.96	12.710	6.08
009	RP	1834	1806									1820	2	19.5	1.07	13.8	0.76	1.52	12.710	9.64
010	RP	1070	1111									1091	2	28.9	2.65	20.4	1.87	3.75	12.710	23.81
011	RP	871	873									872	2	1.6	0.18	1.1	0.13	0.26	12.710	1.66
012	RP	1585	1598									1592	2	9.2	0.58	6.5	0.41	0.82	12.710	5.21
013	RP	4292	4266									4279	2	18.3	0.43	13.0	0.30	0.61	12.710	3.85
014	RP	1633	1608									1620	2	17.1	1.05	12.1	0.75	1.49	12.710	9.47
015	RP	1707	1679									1693	2	19.8	1.17	14.0	0.83	1.65	12.710	10.51

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Glx Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
001	RP	1090	1087	1081	1097	1088	1077	1077	1084	1064	1072	1082	10	9.6	0.89	3.0	0.28	0.56	2.262	0.64
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	1262	1255									1258	2	5.2	0.41	3.7	0.29	0.58	12.710	3.70
009	RP	1299	1369									1334	2	49.7	3.73	35.1	2.63	5.27	12.710	33.48
010	RP	1386	1324									1355	2	43.9	3.24	31.1	2.29	4.59	12.710	29.14
011	RP	1091	1177									1134	2	61.1	5.39	43.2	3.81	7.62	12.710	48.44
012	RP	1176	1298									1237	2	85.7	6.93	60.6	4.90	9.80	12.710	62.25
013	RP	876	876									876	2	0.1	0.01	0.1	0.01	0.02	12.710	0.12
014	RP	1014	1320									1167	2	216.2	18.52	152.8	13.10	26.20	12.710	166.49
015	RP	1136	1343									1240	2	146.6	11.82	103.7	8.36	16.72	12.710	106.27
D-Glx Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
001	RP	162	161	163	165	163	161	161	162	160	160	162	10	1.7	1.03	0.5	0.32	0.65	2.262	0.74
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	205	205									205	2	0.6	0.27	0.4	0.19	0.38	12.710	2.44
009	RP	216	226									221	2	6.9	3.14	4.9	2.22	4.44	12.710	28.21
010	RP	229	218									224	2	7.2	3.23	5.1	2.28	4.56	12.710	29.00
011	RP	181	195									188	2	10.0	5.33	7.1	3.77	7.53	12.710	47.88
012	RP	196	216									206	2	14.5	7.04	10.3	4.98	9.96	12.710	63.27
013	RP	145	145									145	2	0.0	0.01	0.0	0.01	0.01	12.710	0.07
014	RP	166	217									192	2	36.3	18.91	25.6	13.37	26.75	12.710	169.97
015	RP	186	220									203	2	24.1	11.88	17.0	8.40	16.80	12.710	106.77

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Glx		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	0.148	0.148	0.150	0.151	0.150	0.150	0.149	0.149	0.150	0.150	0.150	10	0.0008	0.55	0.0003	0.17	0.35	2.262	0.39
002	RP	0.142	0.139									0.140	2	0.0021	1.49	0.0015	1.06	2.11	12.710	13.42
003	RP	0.144										0.144	1							
004	IE																			
005	IE																			
006[1]	GC_A	0.202										0.202	1							
007[1]	GC_A	0.174										0.174	5	0.0260	14.94	0.0116	6.68	13.37	2.777	18.55
008	RP	0.163	0.163									0.163	2	0.0000	0.00	0.0000	0.00	0.00	12.710	0.00
009	RP	0.166	0.165									0.166	2	0.0010	0.59	0.0007	0.41	0.83	12.710	5.27
010	RP	0.165	0.165									0.165	2	0.0000	0.02	0.0000	0.01	0.02	12.710	0.14
011	RP	0.166	0.166									0.166	2	0.0001	0.06	0.0001	0.04	0.09	12.710	0.56
012	RP	0.166	0.167									0.167	2	0.0002	0.11	0.0001	0.08	0.16	12.710	1.02
013	RP	0.166	0.166									0.166	2	0.0000	0.01	0.0000	0.00	0.01	12.710	0.05
014	RP	0.164	0.165									0.164	2	0.0006	0.39	0.0005	0.28	0.56	12.710	3.54
015	RP	0.164	0.164									0.164	2	0.0001	0.06	0.0001	0.04	0.08	12.710	0.51

[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**

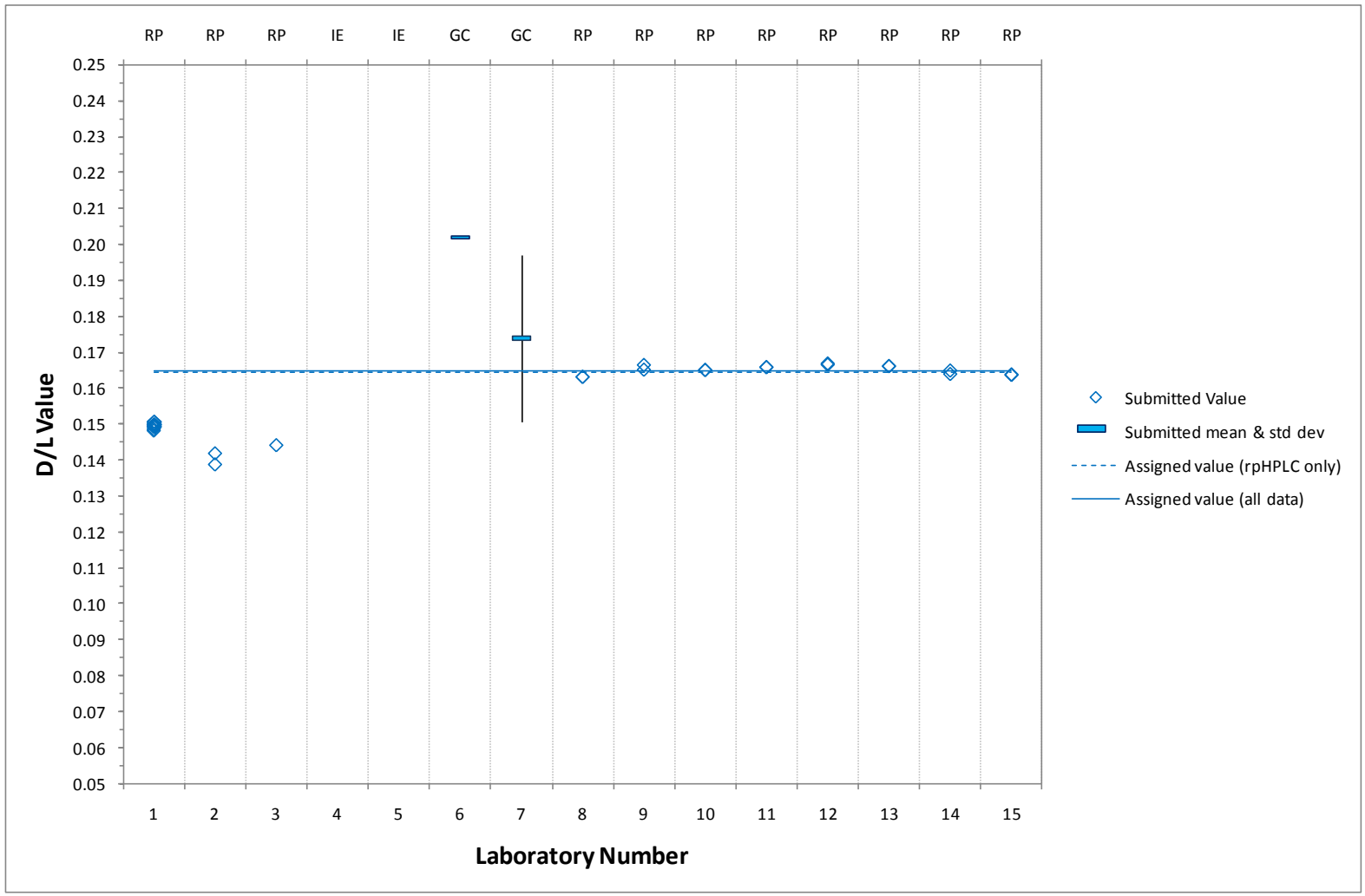


Figure 4.6: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Glutamic Acid / Glutamine** (value of n displayed).

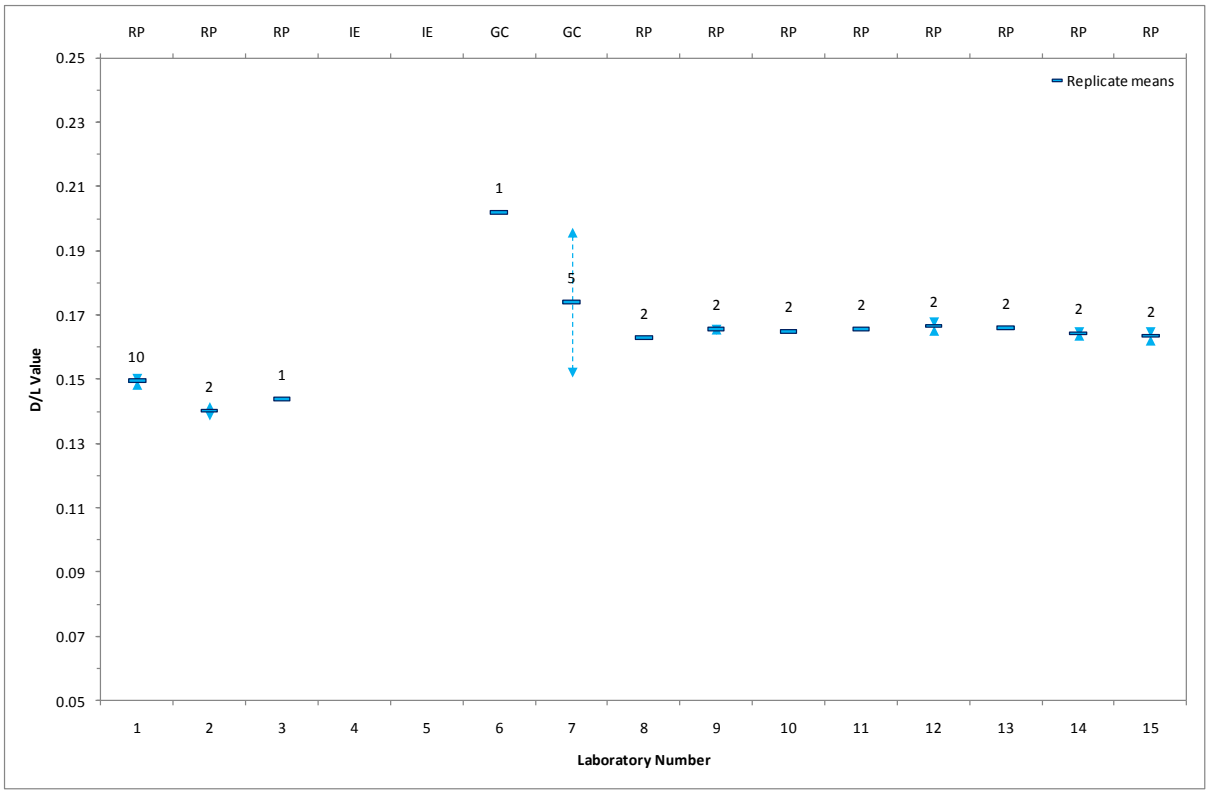


Figure 4.7: Experimental Expanded Uncertainty **(k=$t_{(0.05,df)}$)** of the Mean D/L value for **Glutamic Acid / Glutamine** (value of n displayed).

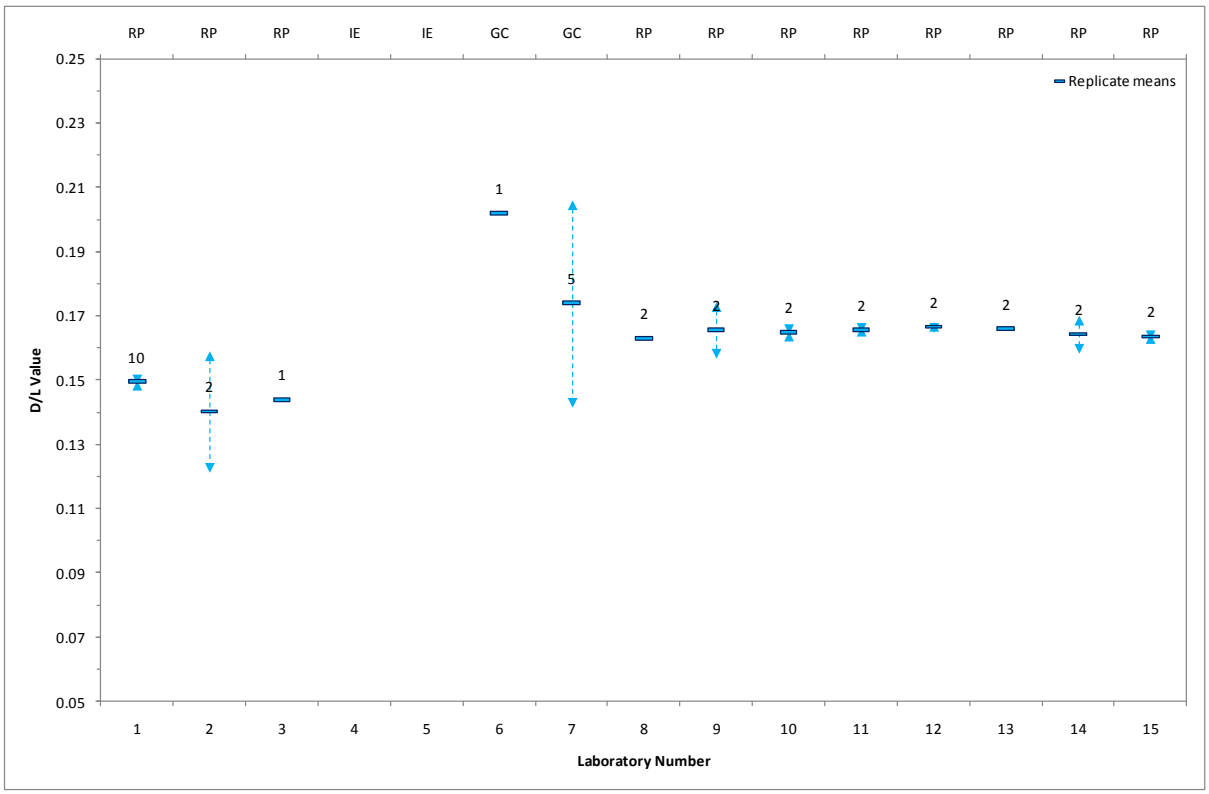


Table 4.8: Summary Statistics for L and D **Serine** Peak Area Data

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)	Exp U% ($k=t_{crit}$)
001	RP	6542	6619	7164	7246	7521	7577	8007	7971	8341	8592	7558	10	685.1	9.06	216.7	2.87	5.73	2.262	6.48
002	RP	795	800									797	2	3.8	0.48	2.7	0.34	0.68	12.710	4.32
003	RP	838										838	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	7440	7376									7408	2	44.8	0.61	31.7	0.43	0.86	12.710	5.44
009	RP	3691	3629									3660	2	44.3	1.21	31.3	0.85	1.71	12.710	10.87
010	RP	2171	2247									2209	2	53.4	2.42	37.7	1.71	3.42	12.710	21.71
011	RP	1752	1762									1757	2	7.1	0.40	5.0	0.29	0.57	12.710	3.63
012	RP	3199	3204									3202	2	3.5	0.11	2.5	0.08	0.15	12.710	0.98
013	RP	8679	8637									8658	2	29.6	0.34	21.0	0.24	0.48	12.710	3.08
014	RP	3292	3230									3261	2	43.9	1.35	31.0	0.95	1.90	12.710	12.09
015	RP	3624	3401									3513	2	157.4	4.48	111.3	3.17	6.34	12.710	40.28
D-Ser peak area		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%			
001	RP	4271	4331	4634	4730	4838	4927	5065	5147	5407	5529	4888	10	416.7	8.53	131.8	2.70	5.39	2.262	6.10
002	RP	524	531									528	2	4.4	0.83	3.1	0.59	1.18	12.710	7.49
003	RP	559										559	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	4996	4971									4983	2	17.9	0.36	12.7	0.25	0.51	12.710	3.24
009	RP	2460	2394									2427	2	46.3	1.91	32.7	1.35	2.70	12.710	17.14
010	RP	1422	1420									1421	2	1.9	0.13	1.3	0.09	0.19	12.710	1.20
011	RP	1131	1165									1148	2	23.6	2.06	16.7	1.46	2.91	12.710	18.50
012	RP	2141	2128									2134	2	9.0	0.42	6.3	0.30	0.59	12.710	3.77
013	RP	5790	5775									5782	2	10.6	0.18	7.5	0.13	0.26	12.710	1.65
014	RP	2185	2089									2137	2	67.9	3.18	48.0	2.25	4.49	12.710	28.56
015	RP	2360	2239									2299	2	85.1	3.70	60.2	2.62	5.23	12.710	33.26

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Ser Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	384	382	387	391	393	389	394	391	388	392	389	10	3.9	1.00	1.2	0.32	0.63	2.262	0.72
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	390	389									390	2	0.8	0.20	0.6	0.14	0.28	12.710	1.80
009	RP	432	451									442	2	13.3	3.00	9.4	2.12	4.25	12.710	26.98
010	RP	461	439									450	2	15.6	3.46	11.0	2.45	4.89	12.710	31.10
011	RP	362	391									377	2	20.9	5.55	14.8	3.92	7.84	12.710	49.85
012	RP	393	431									412	2	27.1	6.57	19.1	4.65	9.29	12.710	59.05
013	RP	292	293									292	2	0.2	0.08	0.2	0.06	0.11	12.710	0.71
014	RP	333	434									384	2	71.4	18.63	50.5	13.17	26.34	12.710	167.40
015	RP	392	443									417	2	35.8	8.58	25.3	6.07	12.14	12.710	77.15
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% ($k=t_{crit}$)
001	RP	251	250	250	256	253	253	249	253	251	252	252	10	1.8	0.73	0.6	0.23	0.46	2.262	0.52
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	262	262									262	2	0.1	0.04	0.1	0.03	0.06	12.710	0.40
009	RP	288	298									293	2	6.7	2.30	4.8	1.63	3.26	12.710	20.71
010	RP	302	277									290	2	17.4	6.01	12.3	4.25	8.50	12.710	53.99
011	RP	234	259									246	2	17.7	7.20	12.5	5.09	10.18	12.710	64.69
012	RP	263	286									275	2	16.6	6.04	11.7	4.27	8.55	12.710	54.31
013	RP	195	196									195	2	0.5	0.24	0.3	0.17	0.34	12.710	2.14
014	RP	221	281									251	2	42.2	16.82	29.8	11.90	23.79	12.710	151.19
015	RP	255	291									273	2	25.6	9.36	18.1	6.62	13.24	12.710	84.15

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Serine		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	0.653	0.654	0.647	0.653	0.643	0.650	0.633	0.646	0.648	0.643	0.647	10	0.0064	0.99	0.0020	0.31	0.63	2.262	0.71
002	RP	0.660	0.663									0.662	2	0.0023	0.35	0.0017	0.25	0.50	12.710	3.17
003	RP	0.667										0.667	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	0.672	0.674									0.673	2	0.0014	0.21	0.0010	0.15	0.30	12.710	1.89
009	RP	0.666	0.660									0.663	2	0.0046	0.70	0.0033	0.49	0.99	12.710	6.27
010	RP	0.655	0.632									0.644	2	0.0164	2.55	0.0116	1.80	3.61	12.710	22.91
011	RP	0.646	0.661									0.653	2	0.0108	1.65	0.0076	1.17	2.34	12.710	14.87
012	RP	0.669	0.664									0.667	2	0.0035	0.53	0.0025	0.37	0.75	12.710	4.75
013	RP	0.667	0.669									0.668	2	0.0011	0.16	0.0008	0.11	0.22	12.710	1.43
014	RP	0.664	0.647									0.655	2	0.0120	1.83	0.0085	1.30	2.59	12.710	16.47
015	RP	0.651	0.658									0.655	2	0.0051	0.78	0.0036	0.55	1.10	12.710	7.02

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

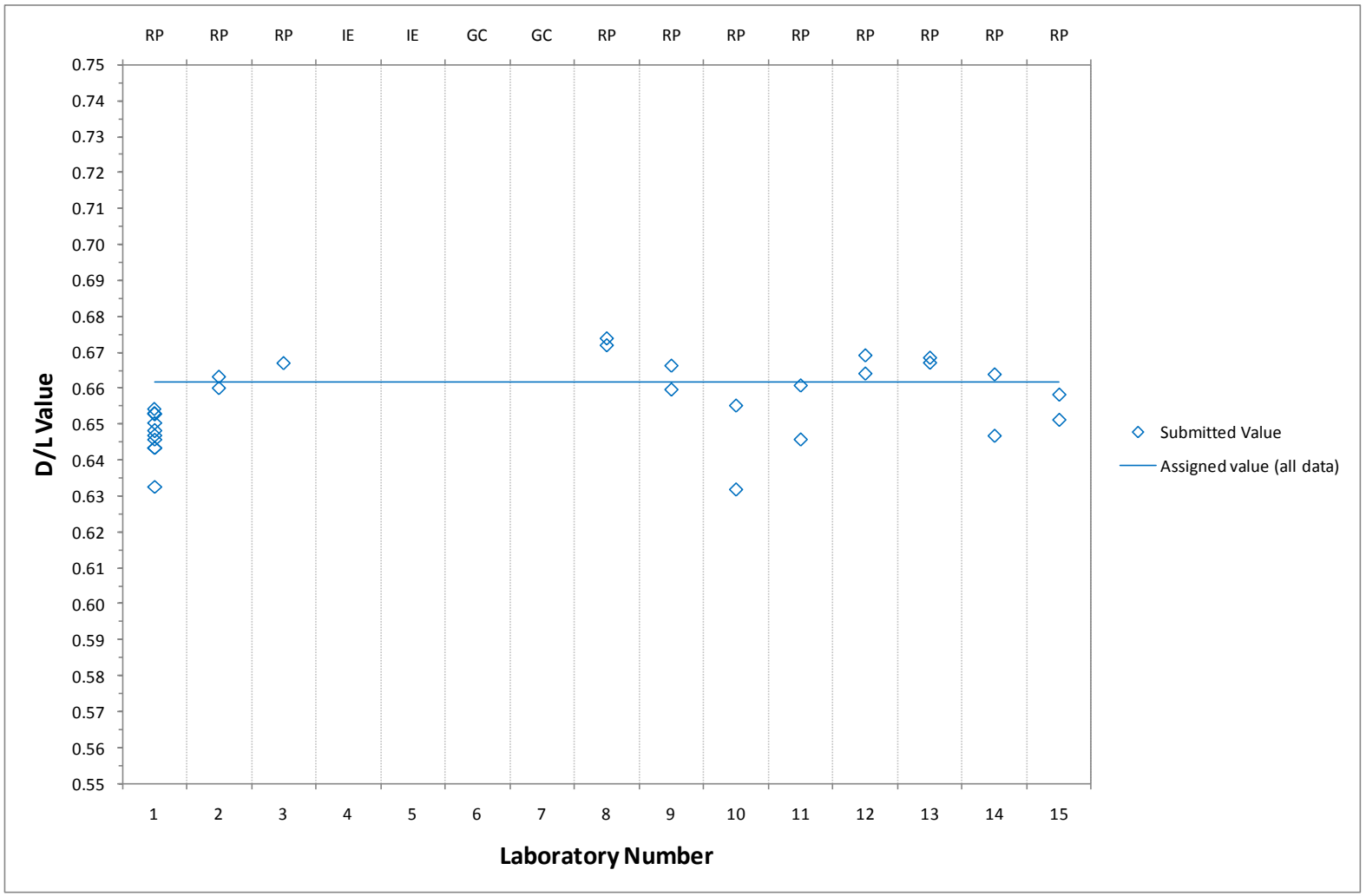


Figure 4.9: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Serine** (value of n displayed).

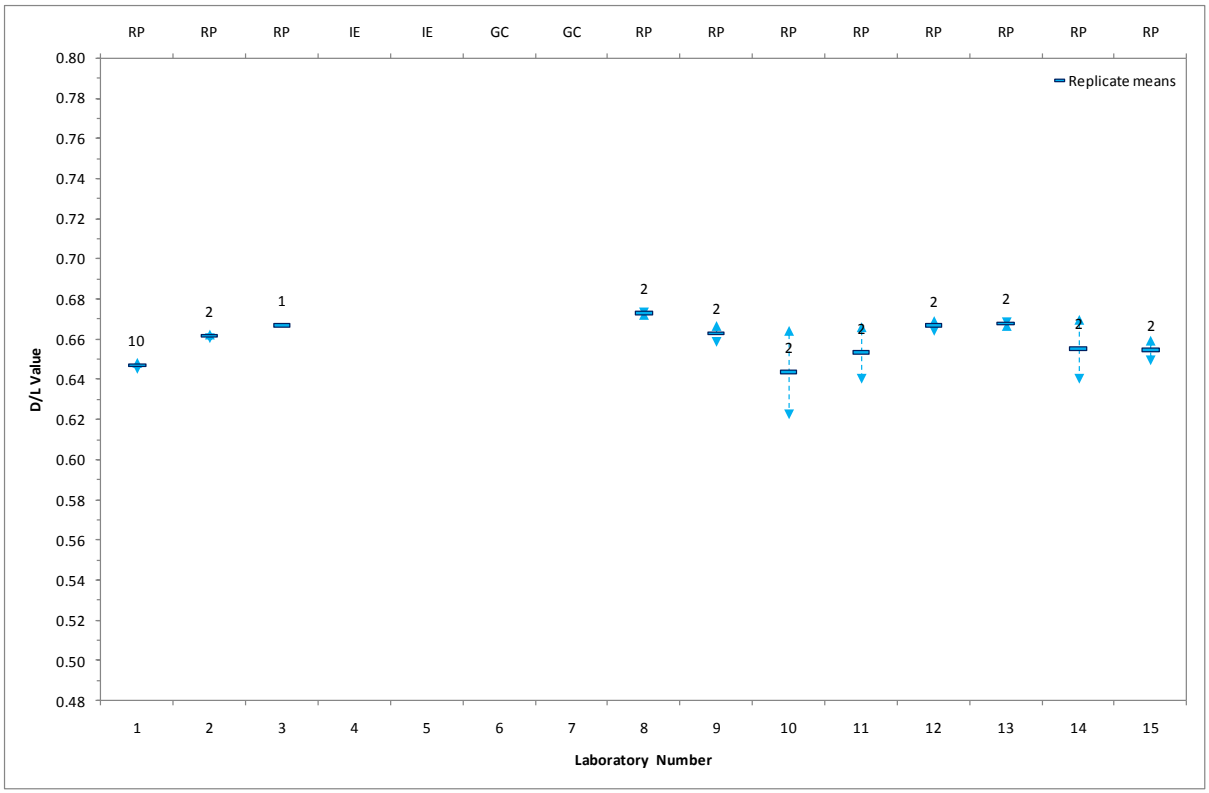


Figure 4.10: Experimental Expanded Uncertainty **($k=t_{(0.05,df)}$)** of the Mean D/L value for **Serine** (value of n displayed).

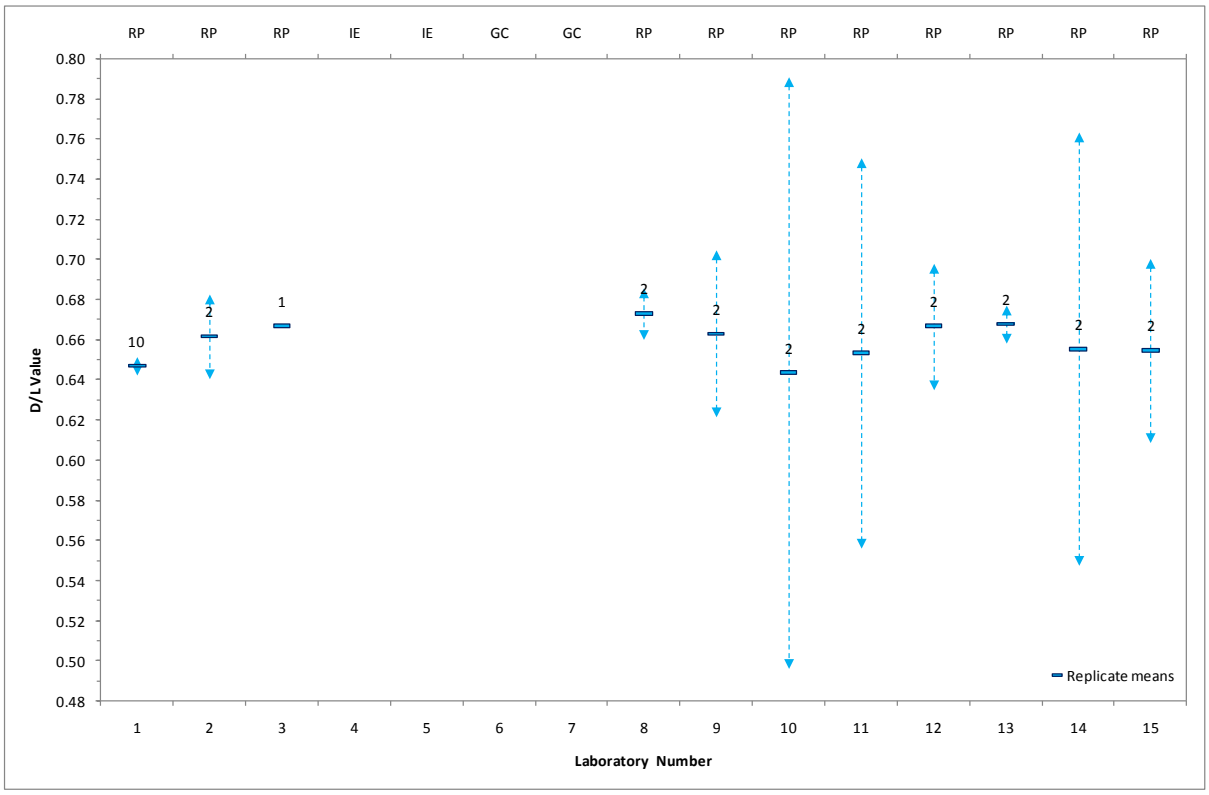


Table 4.11: Summary Statistics for L and D **Arginine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Arg peak area		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP																			
002	RP	550	554									552	2	2.6	0.47	1.8	0.33	0.66	12.710	4.22
003	RP	584										584	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	2879	2695									2787	2	130.3	4.68	92.1	3.31	6.61	12.710	42.02
010	RP	1640	1702									1671	2	43.7	2.62	30.9	1.85	3.70	12.710	23.53
011	RP	1314	1295									1305	2	13.6	1.04	9.6	0.74	1.48	12.710	9.39
012	RP	2524	2412									2468	2	79.4	3.22	56.2	2.28	4.55	12.710	28.92
013	RP	6640	6629									6635	2	7.6	0.12	5.4	0.08	0.16	12.710	1.03
014	RP	2491	2318									2405	2	121.9	5.07	86.2	3.59	7.17	12.710	45.57
015	RP	2674	2559									2617	2	81.3	3.11	57.5	2.20	4.39	12.710	27.92
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% ($k=t_{crit}$)
001	RP																			
002	RP	533	548									540	2	10.1	1.87	7.1	1.32	2.64	12.710	16.78
003	RP	538										538	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	1921	2837									2379	2	648.0	27.24	458.2	19.26	38.52	12.710	244.81
010	RP	985	1687									1336	2	496.7	37.17	351.2	26.28	52.57	12.710	334.06
011	RP	793	947									870	2	108.9	12.51	77.0	8.84	17.69	12.710	112.39
012	RP	1559	1950									1755	2	276.6	15.76	195.6	11.15	22.29	12.710	141.68
013	RP	2197	2664									2430	2	330.2	13.59	233.5	9.61	19.21	12.710	122.11
014	RP	2435	2129									2282	2	216.5	9.49	153.1	6.71	13.42	12.710	85.26
015	RP	2230	1976									2103	2	179.2	8.52	126.7	6.02	12.05	12.710	76.57

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Arg Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP																			
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	332	330									331	2	1.5	0.47	1.1	0.33	0.66	12.710	4.18
010	RP	343	328									335	2	10.9	3.26	7.7	2.30	4.61	12.710	29.28
011	RP	267	283									275	2	11.3	4.10	8.0	2.90	5.80	12.710	36.84
012	RP	305	320									312	2	10.1	3.25	7.2	2.30	4.59	12.710	29.19
013	RP	220	221									221	2	0.7	0.31	0.5	0.22	0.43	12.710	2.75
014	RP	248	307									277	2	41.5	14.95	29.3	10.57	21.14	12.710	134.37
015	RP	285	328									306	2	30.5	9.95	21.6	7.04	14.08	12.710	89.47
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% ($k=t_{crit}$)
001	RP																			
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	221	347									284	2	88.9	31.27	62.9	22.11	44.22	12.710	281.04
010	RP	206	325									265	2	84.0	31.64	59.4	22.37	44.75	12.710	284.37
011	RP	161	207									184	2	32.4	17.59	22.9	12.44	24.88	12.710	158.11
012	RP	189	258									223	2	49.4	22.11	34.9	15.64	31.27	12.710	198.74
013	RP	73	89									81	2	11.3	14.00	8.0	9.90	19.80	12.710	125.85
014	RP	243	282									262	2	27.7	10.56	19.6	7.47	14.93	12.710	94.90
015	RP	238	253									245	2	11.2	4.55	7.9	3.21	6.43	12.710	40.86

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% ($k=t_{crit}$)
001	RP																			
002	RP	0.969	0.988									0.979	2	0.0137	1.40	0.0097	0.99	1.98	12.710	12.56
003	RP	0.921										0.921	1							
004	IE																			
005	IE																			
006[1]	GC																			
007[1]	GC																			
008	RP																			
009	RP	0.667	1.053									0.860	2	0.2727	31.71	0.1928	22.42	44.85	12.710	285.02
010	RP	0.601	0.992									0.796	2	0.2764	34.72	0.1954	24.55	49.10	12.710	312.05
011	RP	0.604	0.732									0.668	2	0.0904	13.54	0.0639	9.58	19.15	12.710	121.70
012	RP	0.618	0.809									0.713	2	0.1350	18.93	0.0955	13.39	26.78	12.710	170.17
013	RP	0.331	0.402									0.366	2	0.0502	13.70	0.0355	9.69	19.38	12.710	123.13
014	RP	0.978	0.918									0.948	2	0.0420	4.43	0.0297	3.13	6.26	12.710	39.79
015	RP	0.834	0.772									0.803	2	0.0435	5.42	0.0308	3.83	7.67	12.710	48.72

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

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

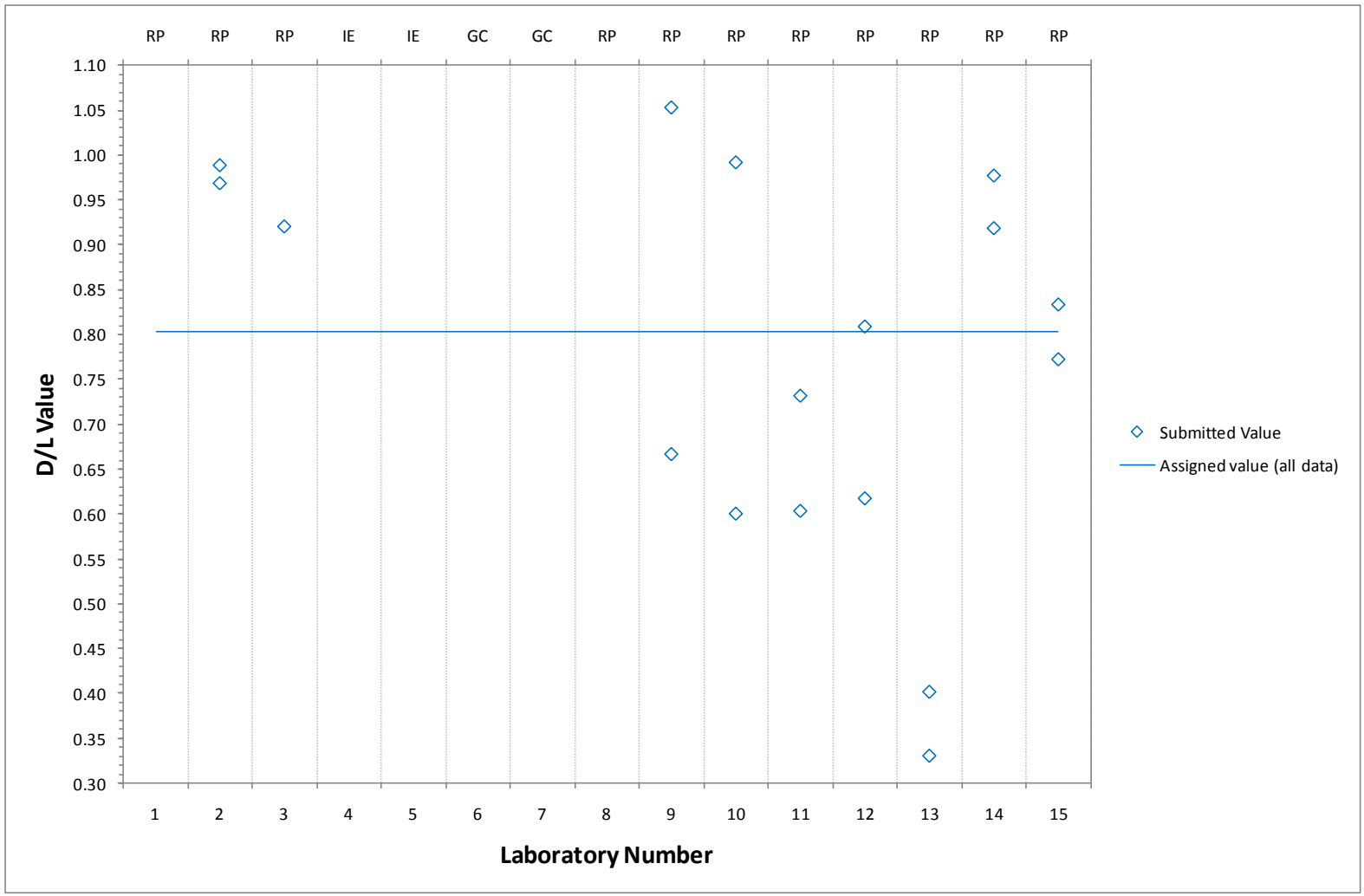


Figure 4.12: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Arginine** (value of n displayed).

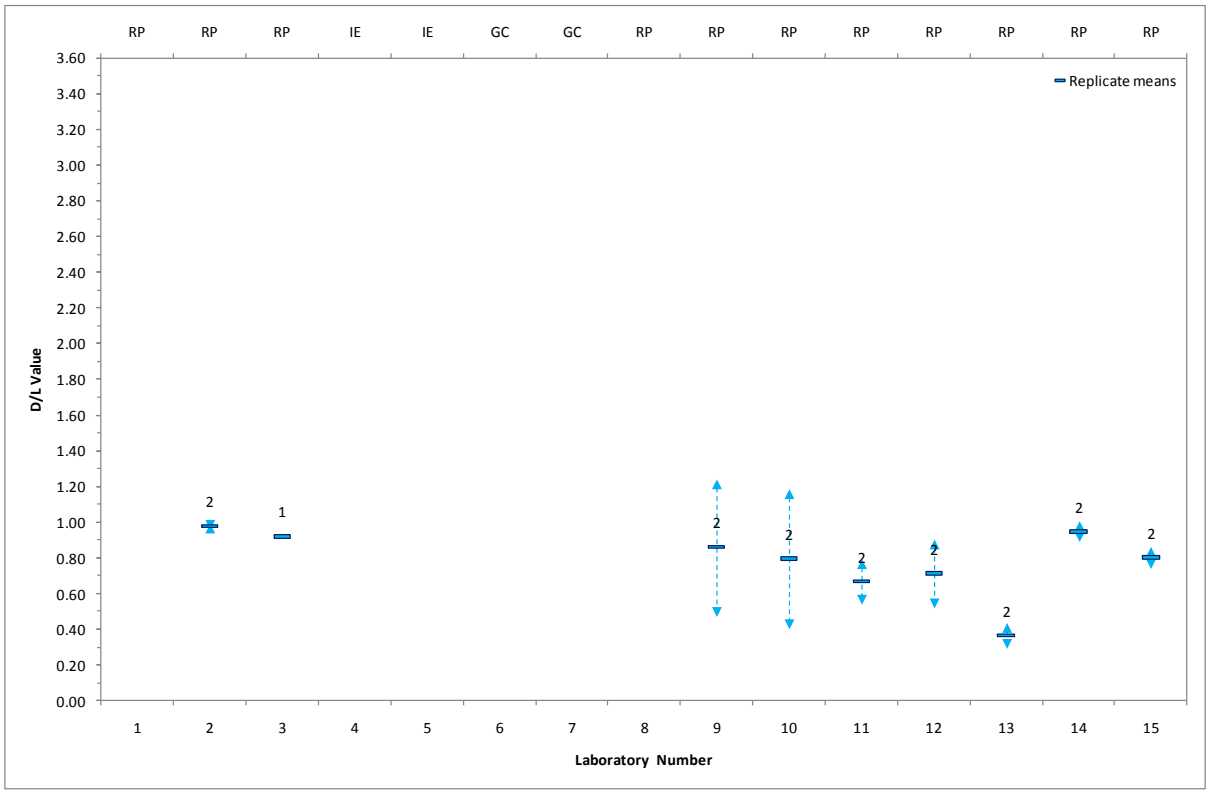


Figure 4.13: Experimental Expanded Uncertainty **(k=$t_{(0.05,df)}$)** of the Mean D/L value for **Arginine** (value of n displayed).

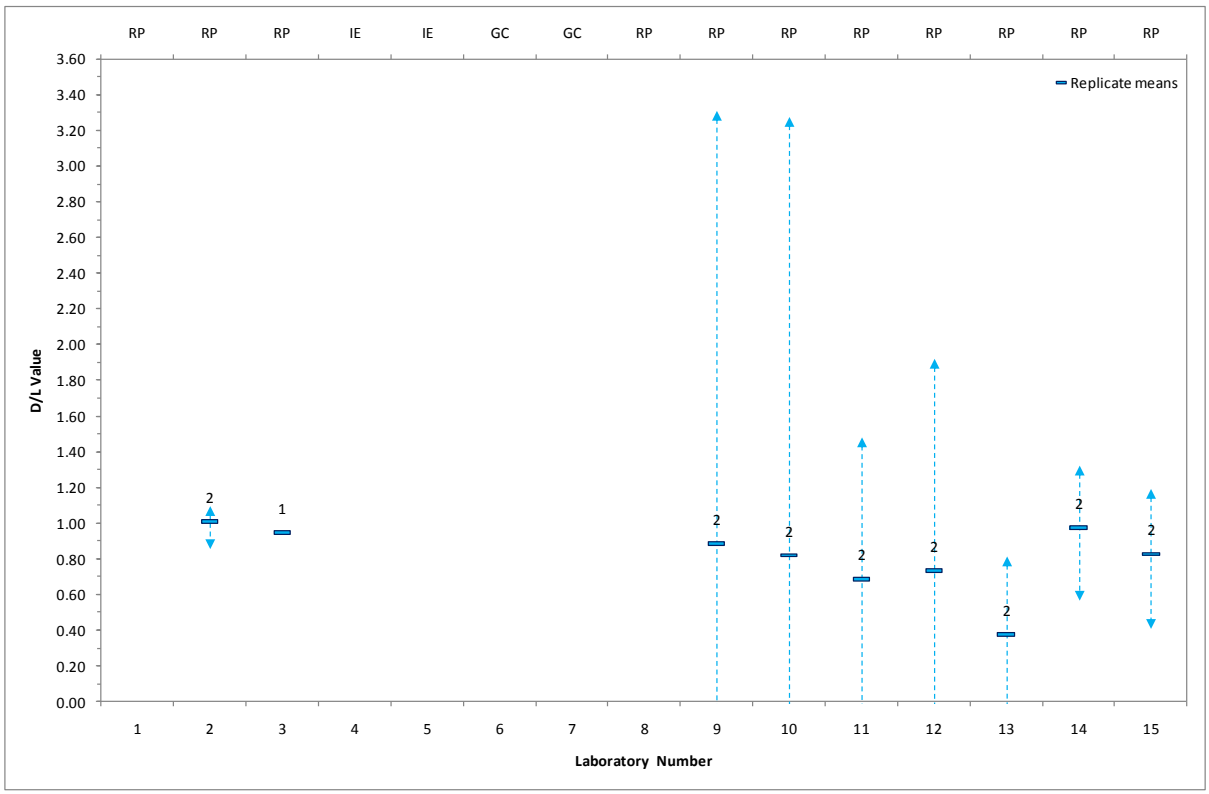


Table 4.14: Summary Statistics for L and D **Alanine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Ala peak area		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	18724	19072	19527	20375	21015	21279	21972	22557	24061	23843	21243	10	1878.7	8.84	594.1	2.80	5.59	2.262	6.33
002	RP	2389	2411									2400	2	15.6	0.65	11.0	0.46	0.92	12.710	5.85
003	RP	2463										2463	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	23300	23205									23253	2	67.4	0.29	47.6	0.20	0.41	12.710	2.60
009	RP	10930	10525									10727	2	286.4	2.67	202.5	1.89	3.78	12.710	23.99
010	RP	6287	6570									6429	2	199.6	3.10	141.1	2.19	4.39	12.710	27.90
011	RP	5099	5050									5074	2	35.3	0.70	24.9	0.49	0.98	12.710	6.25
012	RP	9540	9337									9438	2	144.1	1.53	101.9	1.08	2.16	12.710	13.72
013	RP	24294	24297									24296	2	2.2	0.01	1.6	0.01	0.01	12.710	0.08
014	RP	9764	9435									9599	2	232.7	2.42	164.5	1.71	3.43	12.710	21.79
015	RP	10135	9829									9982	2	217.0	2.17	153.4	1.54	3.07	12.710	19.54
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% ($k=t_{crit}$)
001	RP	5322	5326	5638	5642	5796	5933	6063	6228	6604	6628	5918	10	467.4	7.90	147.8	2.50	5.00	2.262	5.65
002	RP	659	650									654	2	6.1	0.93	4.3	0.66	1.31	12.710	8.34
003	RP	705										705	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	6295	6287									6291	2	5.4	0.09	3.8	0.06	0.12	12.710	0.77
009	RP	3435	3264									3349	2	121.1	3.62	85.6	2.56	5.11	12.710	32.50
010	RP	1918	1950									1934	2	22.9	1.18	16.2	0.84	1.68	12.710	10.65
011	RP	1605	1537									1571	2	48.1	3.06	34.0	2.16	4.33	12.710	27.49
012	RP	3004	2866									2935	2	97.5	3.32	69.0	2.35	4.70	12.710	29.87
013	RP	7286	7278									7282	2	5.2	0.07	3.7	0.05	0.10	12.710	0.64
014	RP	2978	2703									2840	2	194.4	6.84	137.5	4.84	9.68	12.710	61.52
015	RP	3112	2845									2979	2	189.2	6.35	133.8	4.49	8.98	12.710	57.08

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Ala Conc		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
001	RP	1099	1101	1056	1101	1097	1092	1081	1107	1118	1089	1094	10	16.8	1.54	5.3	0.49	0.97	2.262	1.10
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	1223	1225									1224	2	1.4	0.12	1.0	0.08	0.16	12.710	1.04
009	RP	1190	1216									1203	2	18.5	1.54	13.1	1.09	2.18	12.710	13.86
010	RP	1241	1194									1217	2	33.7	2.77	23.9	1.96	3.92	12.710	24.91
011	RP	979	1042									1011	2	45.0	4.45	31.8	3.15	6.29	12.710	39.98
012	RP	1089	1168									1128	2	55.7	4.94	39.4	3.49	6.98	12.710	44.38
013	RP	760	765									763	2	3.3	0.43	2.3	0.30	0.61	12.710	3.86
014	RP	918	1179									1048	2	184.1	17.56	130.2	12.42	24.84	12.710	157.86
015	RP	1019	1189									1104	2	120.2	10.88	85.0	7.70	15.39	12.710	97.81
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% ($k=t_{crit}$)**
001		312	307	305	305	303	305	298	306	307	303	305	10	3.7	1.21	1.2	0.38	0.77	2.262	0.87
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	330	332									331	2	1.1	0.32	0.7	0.23	0.45	12.710	2.87
009	RP	317	320									318	2	1.9	0.59	1.3	0.42	0.84	12.710	5.35
010	RP	321	300									311	2	14.6	4.69	10.3	3.32	6.63	12.710	42.15
011	RP	261	269									265	2	5.5	2.09	3.9	1.48	2.95	12.710	18.75
012	RP	291	304									297	2	9.3	3.14	6.6	2.22	4.44	12.710	28.24
013	RP	193	194									194	2	0.7	0.35	0.5	0.25	0.49	12.710	3.14
014	RP	237	286									262	2	34.5	13.19	24.4	9.33	18.66	12.710	118.56
015	RP	265	292									278	2	18.7	6.72	13.2	4.75	9.50	12.710	60.37

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Ala		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	0.284	0.279	0.289	0.277	0.276	0.279	0.276	0.276	0.274	0.278	0.279	10	0.0044	1.58	0.0014	0.50	1.00	2.262	1.13
002	RP	0.276	0.270									0.273	2	0.0043	1.58	0.0030	1.12	2.23	12.710	14.19
003	RP	0.286										0.286	1							
004	IE																			
005	IE																			
006[1]	GC_A	0.246										0.246	1							
007[1]	GC_A	0.265										0.265	7	0.0090	3.40	0.0034	1.28	2.57	2.447	3.14
008	RP	0.270	0.271									0.271	2	0.0007	0.26	0.0005	0.18	0.37	12.710	2.35
009	RP	0.266	0.263									0.265	2	0.0025	0.95	0.0018	0.67	1.34	12.710	8.51
010	RP	0.258	0.252									0.255	2	0.0049	1.92	0.0035	1.36	2.71	12.710	17.25
011	RP	0.267	0.258									0.262	2	0.0062	2.36	0.0044	1.67	3.34	12.710	21.24
012	RP	0.267	0.260									0.263	2	0.0047	1.80	0.0033	1.27	2.54	12.710	16.15
013	RP	0.254	0.254									0.254	2	0.0002	0.08	0.0001	0.06	0.11	12.710	0.72
014	RP	0.258	0.243									0.251	2	0.0111	4.42	0.0078	3.13	6.26	12.710	39.76
015	RP	0.260	0.245									0.253	2	0.0106	4.18	0.0075	2.96	5.91	12.710	37.57

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

GC_A= derived using peak area

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

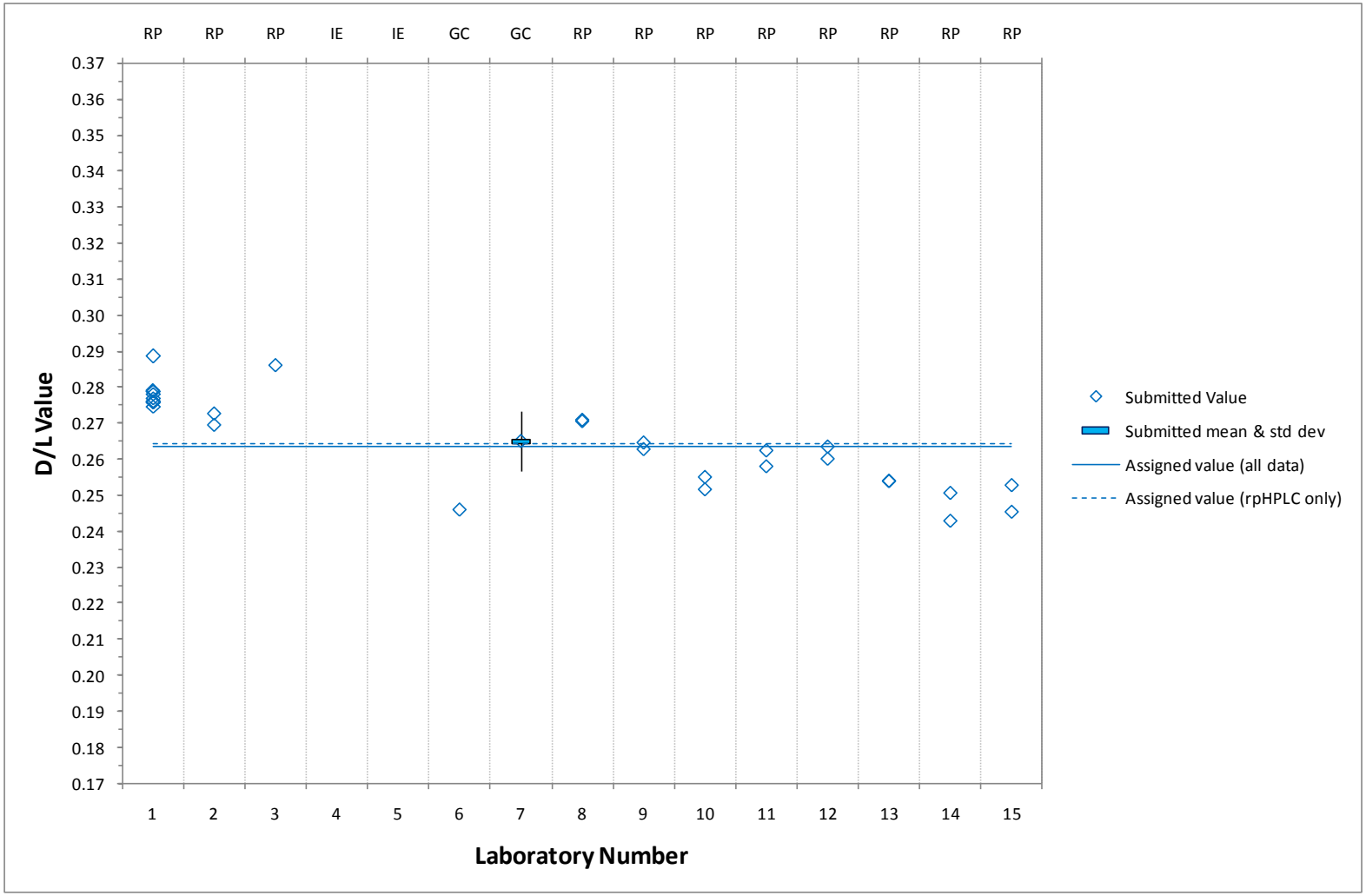


Figure 4.15: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Alanine** (value of n displayed).

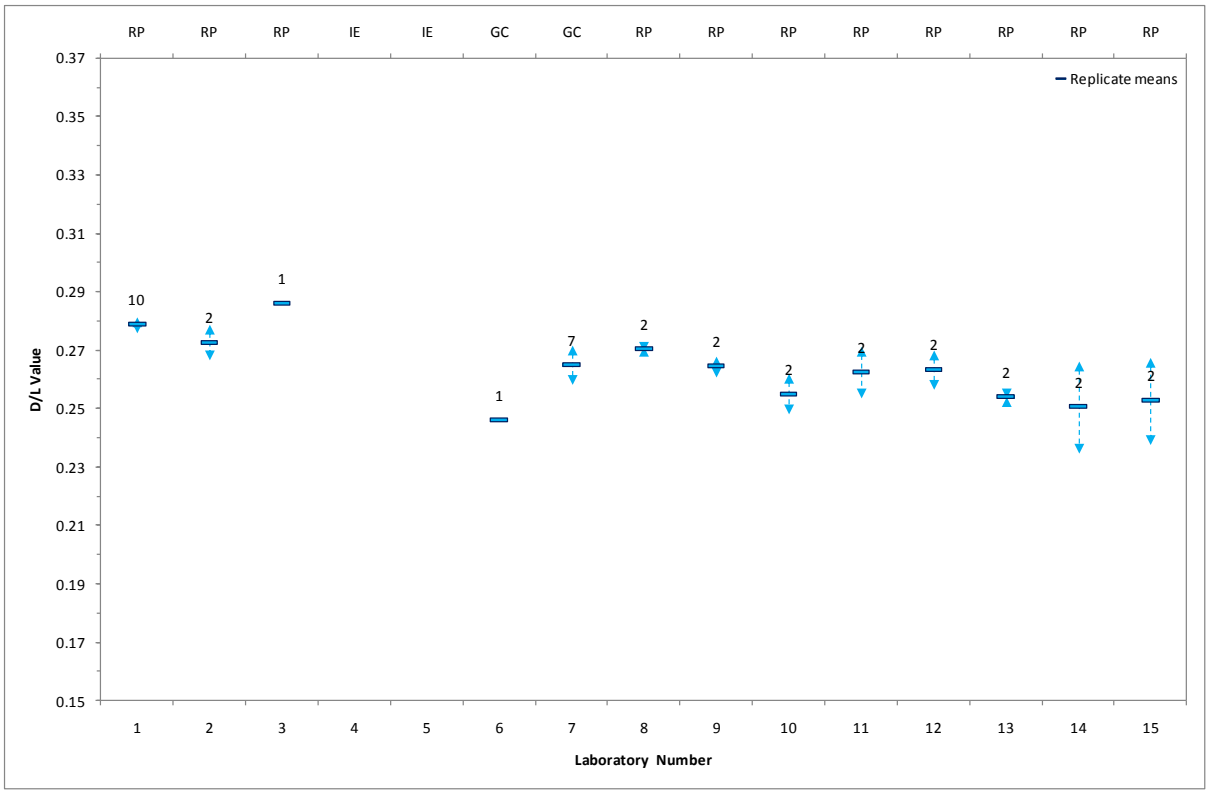


Figure 4.16: Experimental Expanded Uncertainty **($k=t_{(0.05,df)}$)** of the Mean D/L value for **Alanine** (value of n displayed).

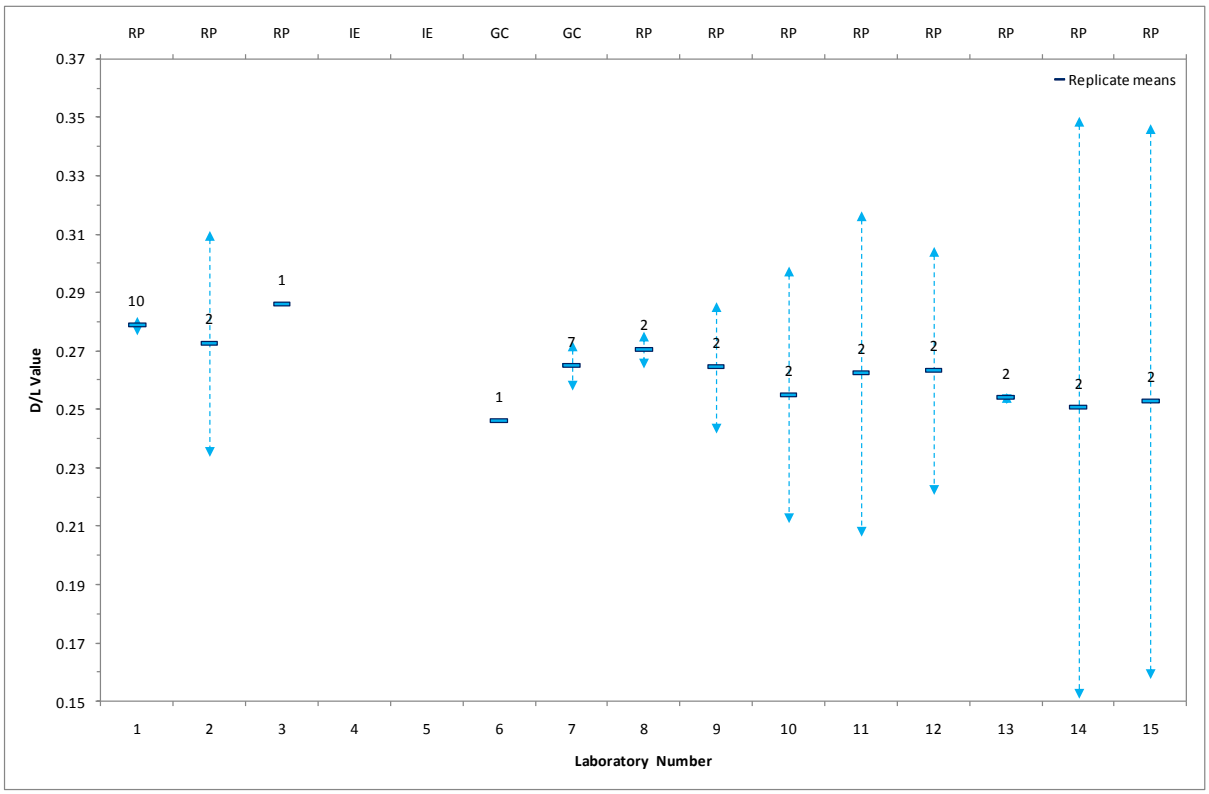


Table 4.17: Summary Statistics for L and D **Valine** Peak Area / Height Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Val peak area		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	13704	13998	13737	14595	15182	15652	16125	16533	17442	17819	15479	10	1495.9	9.66	473.1	3.06	6.11	2.262	6.91
002	RP	1726	1734									1730	2	5.3	0.31	3.7	0.22	0.43	12.710	2.75
003	RP	1777										1777	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	16972	16874									16923	2	69.2	0.41	48.9	0.29	0.58	12.710	3.68
009	RP	8286	8337									8311	2	35.8	0.43	25.3	0.30	0.61	12.710	3.87
010	RP	4721	5059									4890	2	239.0	4.89	169.0	3.46	6.91	12.710	43.93
011	RP	3815	3821									3818	2	4.3	0.11	3.1	0.08	0.16	12.710	1.02
012	RP	7392	7400									7396	2	5.3	0.07	3.8	0.05	0.10	12.710	0.65
013	RP	16355	16155									16255	2	141.2	0.87	99.9	0.61	1.23	12.710	7.81
014	RP	7288	7158									7223	2	92.3	1.28	65.3	0.90	1.81	12.710	11.49
015	RP	7783	7715									7749	2	47.8	0.62	33.8	0.44	0.87	12.710	5.55
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% ($k=t_{crit}$)
001	RP	1815	1862	1931	2002	2080	2178	2243	2353	2475	2546	2148	10	254.4	11.84	80.5	3.75	7.49	2.262	8.47
002	RP	242	246									244	2	2.3	0.96	1.7	0.68	1.35	12.710	8.60
003	RP	257										257	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	2320	2314									2317	2	4.2	0.18	2.9	0.13	0.25	12.710	1.61
009	RP	1217	1202									1209	2	10.6	0.88	7.5	0.62	1.24	12.710	7.87
010	RP	649	678									663	2	20.5	3.10	14.5	2.19	4.38	12.710	27.82
011	RP	514	523									519	2	5.9	1.13	4.1	0.80	1.60	12.710	10.17
012	RP	1101	1007									1054	2	66.8	6.34	47.2	4.48	8.96	12.710	56.95
013	RP	2559	2537									2548	2	15.6	0.61	11.0	0.43	0.87	12.710	5.50
014	RP	1314	1069									1192	2	173.2	14.53	122.5	10.28	20.55	12.710	130.61
015	RP	1123	1046									1085	2	54.1	4.99	38.2	3.53	7.05	12.710	44.82
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	15.13	15.16									15.15	2	0.0269	0.18	0.0190	0.13	0.25	12.710	1.59
005	IE	12.82	14.82									13.82	2	1.4086	10.19	0.9960	7.21	14.41	12.710	91.60

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Val Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	805	808	743	789	792	803	793	811	810	814	797	10	20.9	2.63	6.6	0.83	1.66	2.262	1.88
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	891	891									891	2	0.0	0.00	0.0	0.00	0.01	12.710	0.04
009	RP	832	888									860	2	39.9	4.64	28.2	3.28	6.56	12.710	41.71
010	RP	859	847									853	2	8.4	0.99	6.0	0.70	1.40	12.710	8.88
011	RP	675	727									701	2	36.9	5.26	26.1	3.72	7.43	12.710	47.24
012	RP	778	853									816	2	53.3	6.53	37.7	4.62	9.24	12.710	58.72
013	RP	472	469									470	2	2.1	0.45	1.5	0.32	0.63	12.710	4.03
014	RP	632	824									728	2	136.1	18.69	96.2	13.22	26.43	12.710	167.99
015	RP	722	861									791	2	98.3	12.43	69.5	8.79	17.58	12.710	111.70
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% ($k=t_{crit}$)
001	RP	107	107	104	108	109	112	110	115	115	116	110	10	4.1	3.70	1.3	1.17	2.34	2.262	2.65
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	122	122									122	2	0.3	0.23	0.2	0.16	0.32	12.710	2.03
009	RP	110	115									113	2	3.8	3.34	2.7	2.36	4.72	12.710	29.98
010	RP	106	102									104	2	2.9	2.78	2.1	1.97	3.93	12.710	24.99
011	RP	82	90									86	2	5.4	6.27	3.8	4.44	8.87	12.710	56.38
012	RP	104	105									104	2	0.1	0.13	0.1	0.09	0.18	12.710	1.13
013	RP	67	66									66	2	0.1	0.19	0.1	0.14	0.27	12.710	1.72
014	RP	103	111									107	2	5.9	5.49	4.1	3.88	7.77	12.710	49.37
015	RP	94	105									99	2	8.0	8.08	5.7	5.71	11.43	12.710	72.62

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Valine		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	0.132	0.133	0.141	0.137	0.137	0.139	0.139	0.142	0.142	0.143	0.139	10	0.0037	2.64	0.0012	0.84	1.67	2.262	1.89
002	RP	0.140	0.142									0.141	2	0.0009	0.65	0.0006	0.46	0.92	12.710	5.85
003	RP	0.144										0.144	1							
004	IE																			
005	IE																			
006[1]	GC_A	0.137										0.137	1							
007[1]	GC_A	0.109										0.109	9	0.0060	5.50	0.0020	1.83	3.67	2.306	4.23
008	RP	0.137	0.137									0.137	2	0.0000	0.00	0.0000	0.00	0.00	12.710	0.00
009	RP	0.132	0.130									0.131	2	0.0017	1.31	0.0012	0.92	1.85	12.710	11.74
010	RP	0.124	0.121									0.122	2	0.0022	1.79	0.0016	1.27	2.54	12.710	16.12
011	RP	0.121	0.123									0.122	2	0.0012	1.02	0.0009	0.72	1.44	12.710	9.15
012	RP	0.134	0.123									0.128	2	0.0082	6.41	0.0058	4.53	9.06	12.710	57.59
013	RP	0.141	0.141									0.141	2	0.0004	0.26	0.0003	0.18	0.36	12.710	2.31
014	RP	0.162	0.135									0.149	2	0.0197	13.27	0.0139	9.38	18.76	12.710	119.23
015	RP	0.130	0.122									0.126	2	0.0055	4.37	0.0039	3.09	6.18	12.710	39.28

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

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

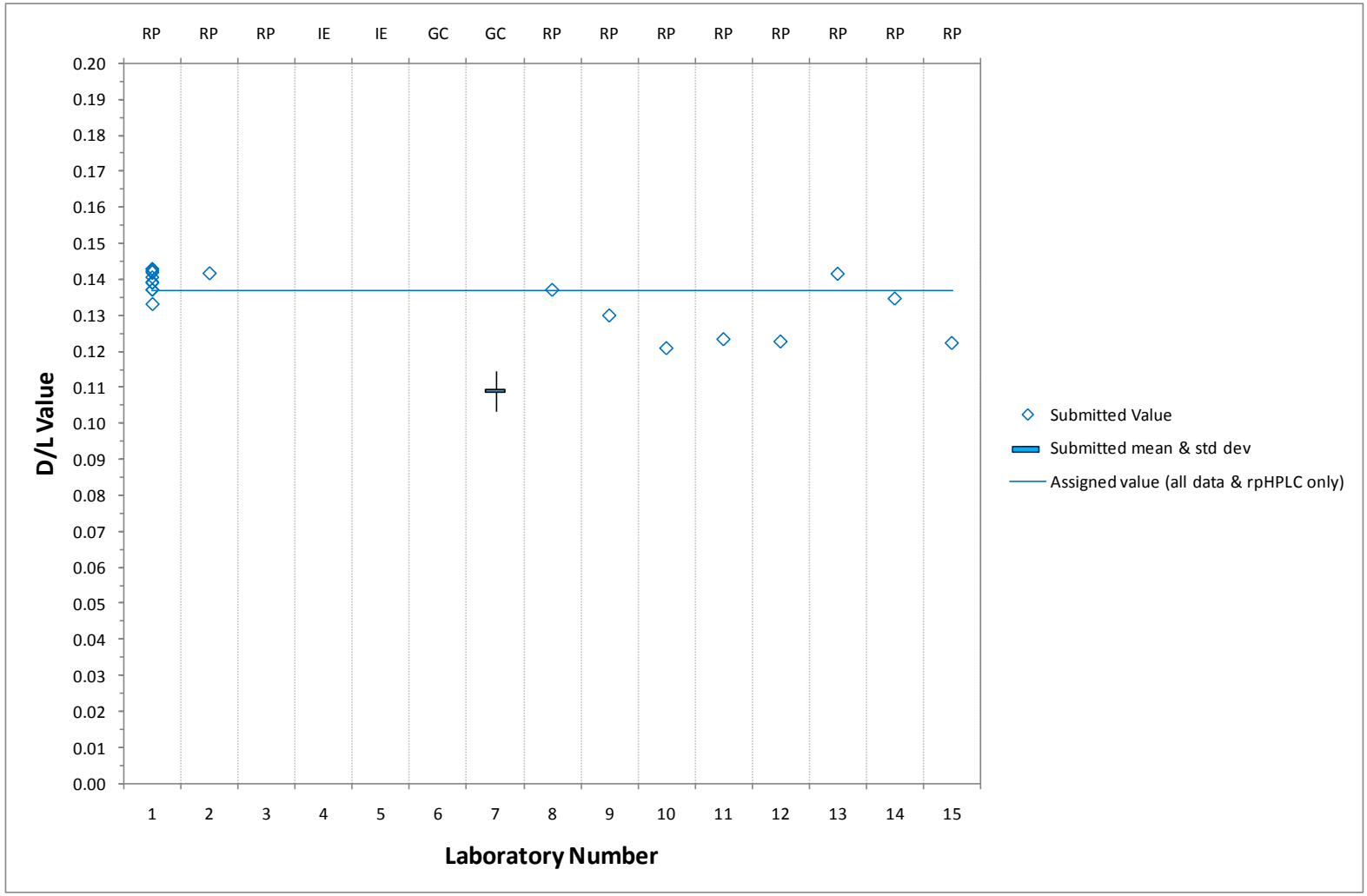


Figure 4.18: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Valine** (value of n displayed).

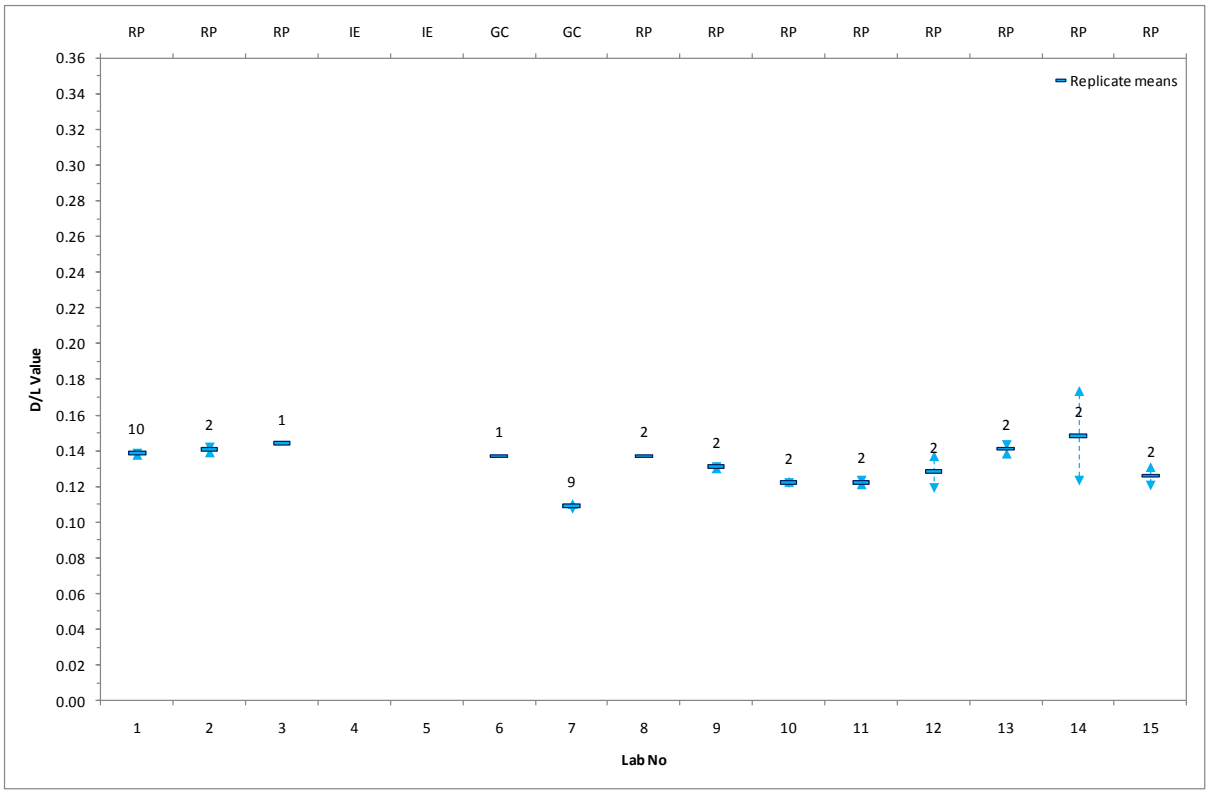


Figure 4.19: Experimental Expanded Uncertainty **($k=t_{(0.05,df)}$)** of the Mean D/L value for **Valine** (value of n displayed).

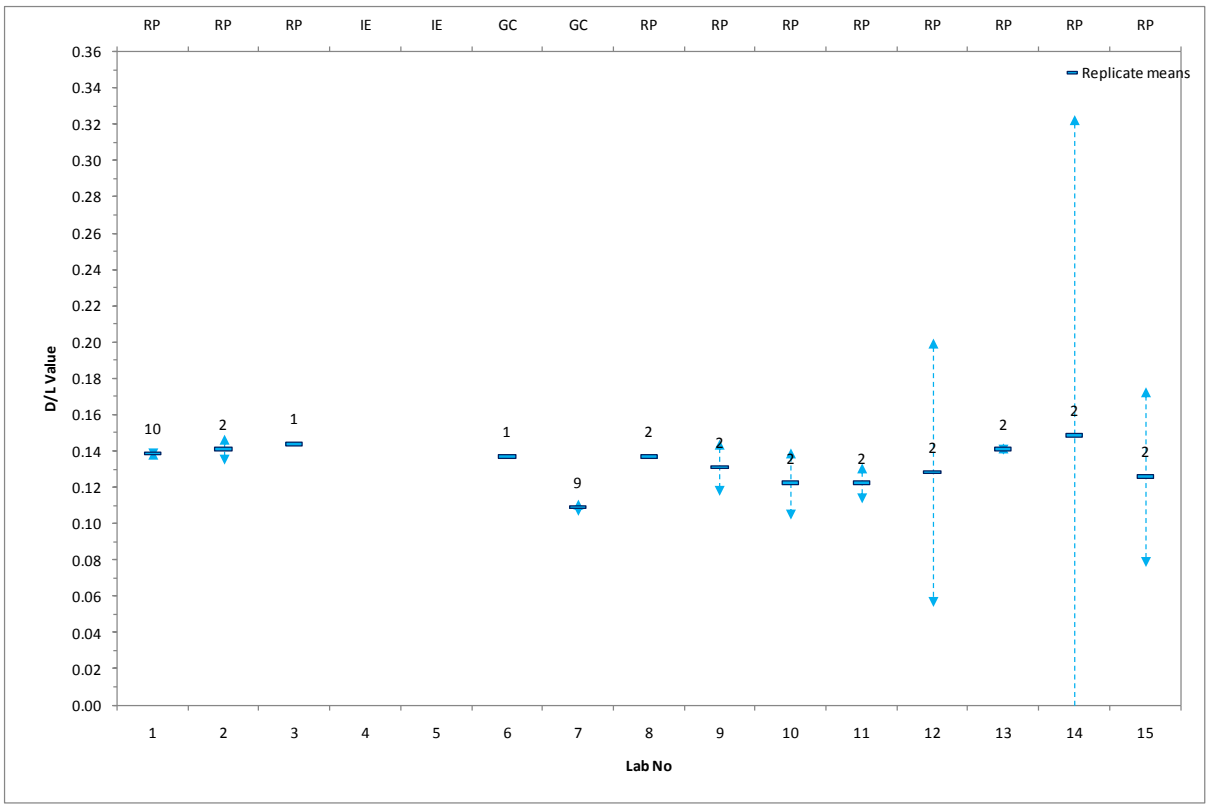


Table 4.20: Summary Statistics for L and D **Phenylalanine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Phe peak area		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	4457	4557	4778	4827	4984	5093	5285	5337	5572	5668	5056	10	410.4	8.12	129.8	2.57	5.13	2.262	5.81
002	RP	570	577									573	2	5.4	0.94	3.8	0.67	1.33	12.710	8.48
003	RP	599										599	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	4527	4521									4524	2	4.1	0.09	2.9	0.06	0.13	12.710	0.80
009	RP	2654	2657									2656	2	2.0	0.07	1.4	0.05	0.11	12.710	0.67
010	RP	1463	1569									1516	2	74.7	4.93	52.8	3.48	6.97	12.710	44.28
011	RP	1161	1158									1159	2	2.4	0.20	1.7	0.14	0.29	12.710	1.83
012	RP	2304	2257									2280	2	33.6	1.47	23.7	1.04	2.08	12.710	13.22
013	RP	5941	5910									5926	2	21.7	0.37	15.4	0.26	0.52	12.710	3.30
014	RP	2305	2238									2271	2	47.4	2.09	33.5	1.48	2.95	12.710	18.76
015	RP	2408	2322									2365	2	61.4	2.60	43.4	1.84	3.67	12.710	23.34
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% ($k=t_{crit}$)
001	RP	1430	1482	1383	1434	1458	1540	1601	1679	1523	1492	1502	10	87.8	5.84	27.8	1.85	3.70	2.262	4.18
002	RP	171	172									171	2	0.9	0.54	0.7	0.38	0.76	12.710	4.82
003	RP	195										195	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	1564	1548									1556	2	11.1	0.71	7.8	0.50	1.01	12.710	6.40
009	RP	821	815									818	2	3.7	0.45	2.6	0.32	0.64	12.710	4.04
010	RP	442	468									455	2	19.0	4.18	13.5	2.96	5.91	12.710	37.58
011	RP	356	352									354	2	2.7	0.77	1.9	0.54	1.09	12.710	6.90
012	RP	694	693									693	2	0.5	0.07	0.3	0.05	0.09	12.710	0.59
013	RP	1875	1851									1863	2	17.5	0.94	12.4	0.66	1.33	12.710	8.45
014	RP	762	641									702	2	85.7	12.21	60.6	8.64	17.27	12.710	109.77
015	RP	715	690									703	2	17.4	2.48	12.3	1.75	3.51	12.710	22.30

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Phe Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	262	263	258	261	260	261	260	262	259	259	260	10	1.5	0.59	0.5	0.19	0.37	2.262	0.42
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	238	239									238	2	0.8	0.32	0.5	0.22	0.45	12.710	2.83
009	RP	291	309									300	2	12.8	4.29	9.1	3.03	6.06	12.710	38.52
010	RP	291	287									289	2	2.7	0.95	1.9	0.67	1.34	12.710	8.52
011	RP	224	240									232	2	11.5	4.94	8.1	3.49	6.99	12.710	44.40
012	RP	265	284									274	2	13.7	4.99	9.7	3.53	7.06	12.710	44.87
013	RP	187	187									187	2	0.1	0.05	0.1	0.04	0.08	12.710	0.49
014	RP	218	281									250	2	44.7	17.90	31.6	12.65	25.31	12.710	160.83
015	RP	244	283									263	2	27.5	10.46	19.5	7.40	14.79	12.710	94.02
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% ($k=t_{crit}$)
001	RP	84	86	75	77	76	79	79	82	71	68	78	10	5.5	7.13	1.8	2.25	4.51	2.262	5.10
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	82	82									82	2	0.3	0.31	0.2	0.22	0.44	12.710	2.76
009	RP	90	95									92	2	3.5	3.76	2.5	2.66	5.32	12.710	33.81
010	RP	88	86									87	2	1.5	1.69	1.0	1.20	2.40	12.710	15.23
011	RP	69	73									71	2	3.1	4.38	2.2	3.09	6.19	12.710	39.33
012	RP	80	87									83	2	5.3	6.40	3.8	4.52	9.05	12.710	57.48
013	RP	59	59									59	2	0.3	0.52	0.2	0.37	0.73	12.710	4.67
014	RP	72	81									76	2	6.0	7.83	4.2	5.53	11.07	12.710	70.35
015	RP	72	84									78	2	8.3	10.58	5.8	7.48	14.96	12.710	95.06

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Phe		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
001	RP	0.321	0.325	0.289	0.297	0.293	0.302	0.303	0.315	0.273	0.263	0.298	10	0.0197	6.61	0.0062	2.09	4.18	2.262	4.73
002	RP	0.300	0.298									0.299	2	0.0012	0.41	0.0009	0.29	0.58	12.710	3.66
003	RP	0.326										0.326	1							
004	IE																			
005	IE																			
006[1]	GC_A	0.297										0.297	1							
007[1]	GC_A	0.280										0.280	10	0.0300	10.71	0.0095	3.39	6.78	2.262	7.66
008	RP	0.345	0.342									0.344	2	0.0021	0.62	0.0015	0.44	0.87	12.710	5.55
009	RP	0.309	0.307									0.308	2	0.0016	0.52	0.0011	0.37	0.74	12.710	4.71
010	RP	0.302	0.299									0.300	2	0.0022	0.75	0.0016	0.53	1.06	12.710	6.71
011	RP	0.306	0.304									0.305	2	0.0017	0.56	0.0012	0.40	0.80	12.710	5.08
012	RP	0.301	0.307									0.304	2	0.0043	1.41	0.0030	0.99	1.99	12.710	12.63
013	RP	0.316	0.313									0.314	2	0.0018	0.57	0.0013	0.41	0.81	12.710	5.15
014	RP	0.331	0.286									0.309	2	0.0313	10.14	0.0221	7.17	14.34	12.710	91.13
015	RP	0.297	0.297									0.297	2	0.0003	0.12	0.0002	0.08	0.16	12.710	1.04

[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**

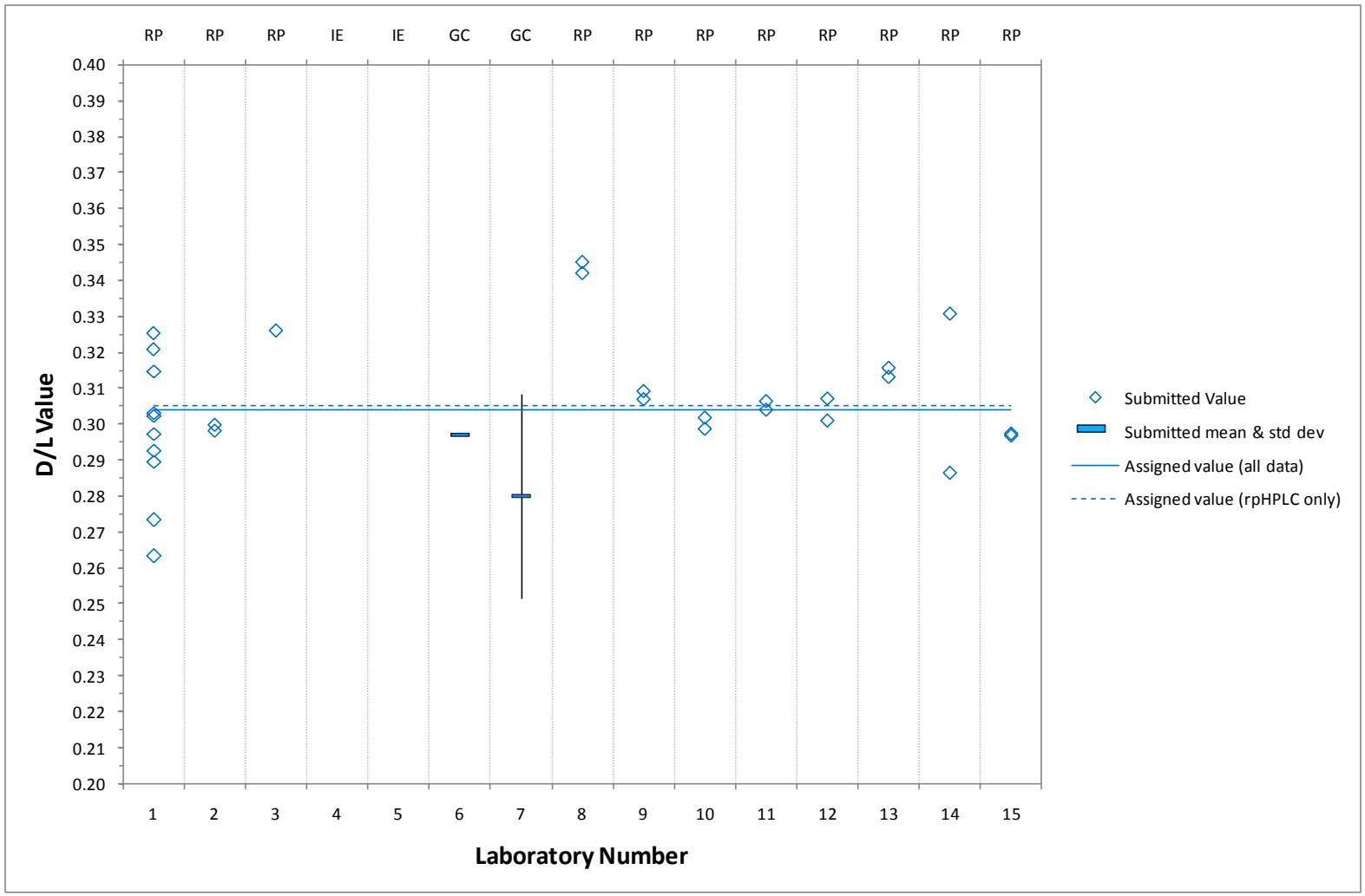


Figure 4.21: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Phenylalanine** (value of n displayed).

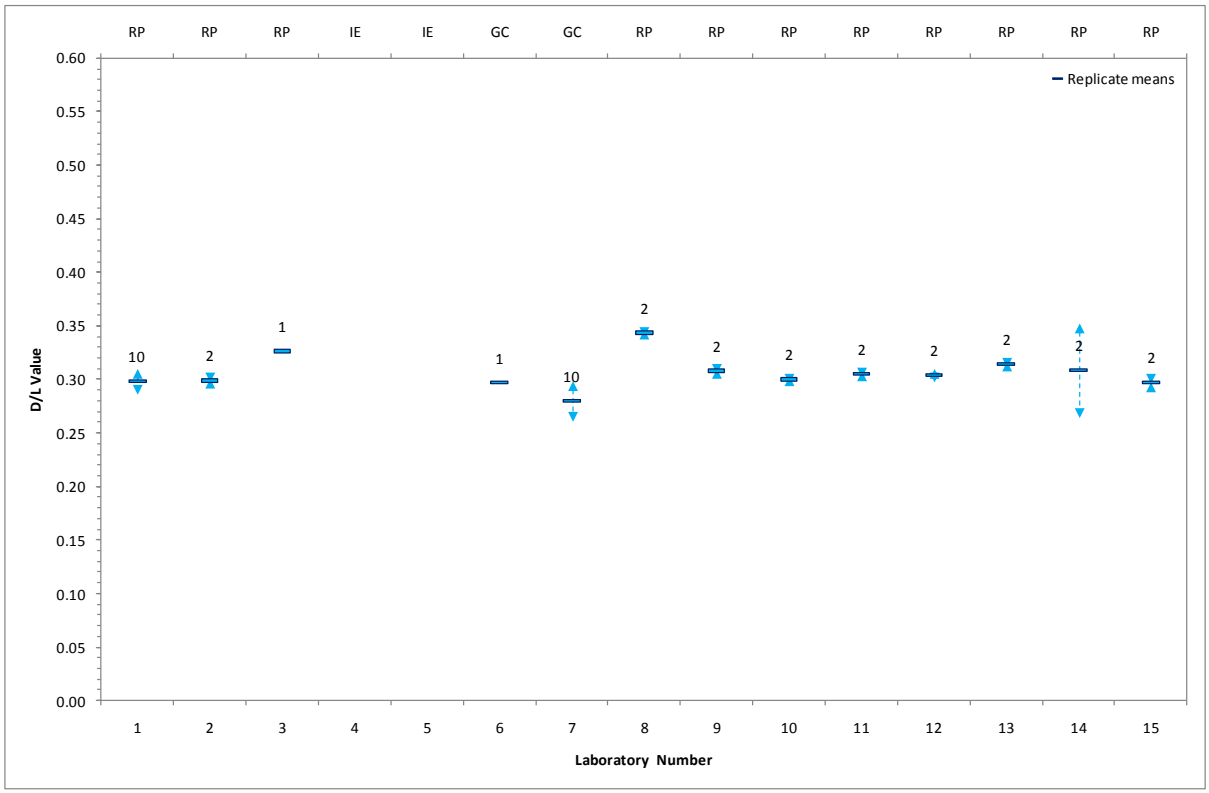


Figure 4.22: Experimental Expanded Uncertainty **($k=t_{(0.05,df)}$)** of the Mean D/L value for **Phenylalanine** (value of n displayed).

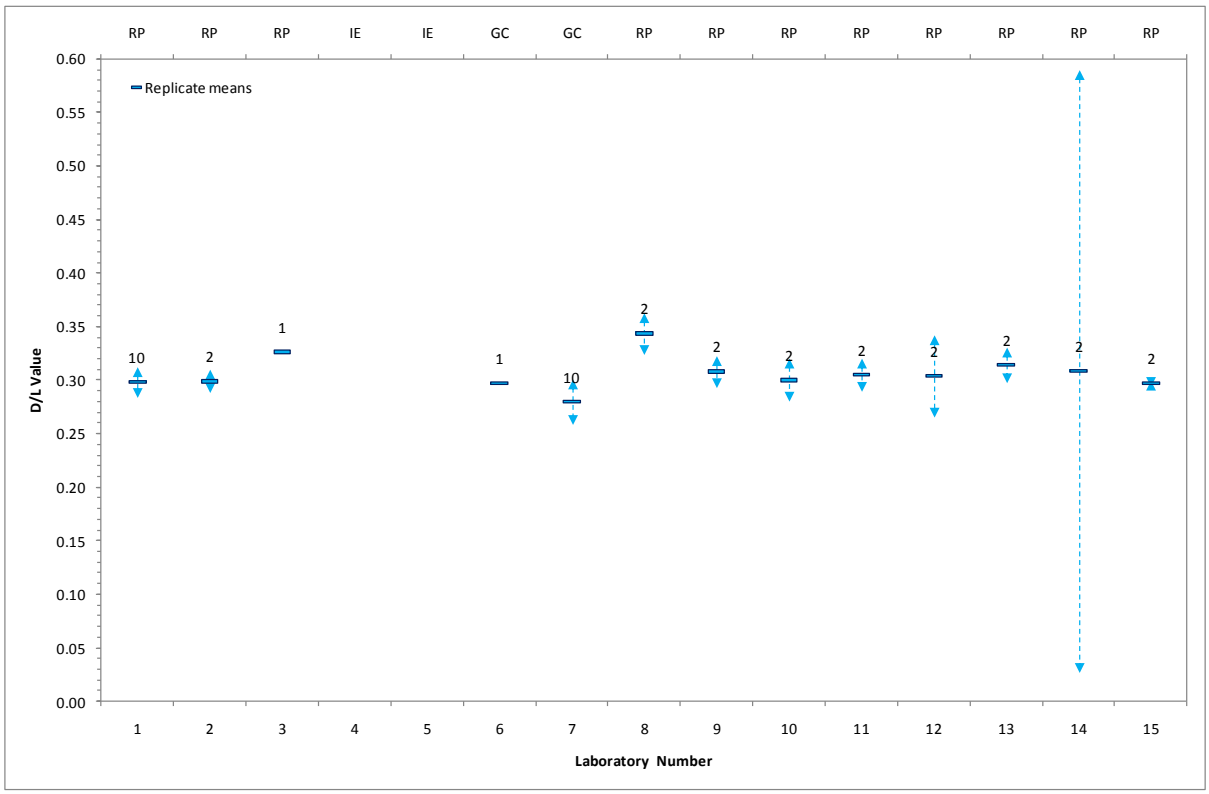


Table 4.23: Summary Statistics for **D-Alloisoleucine/L-Isoleucine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Ile peak area		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	5332	5347	5356	5619	5840	6025	6218	6382	6640	6787	5955	10	543.7	9.13	171.9	2.89	5.77	2.262	6.53
002	RP	614	613									613	2	1.0	0.16	0.7	0.12	0.23	12.710	1.47
003	RP	649										649	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	5716	5686									5701	2	21.3	0.37	15.0	0.26	0.53	12.710	3.35
009	RP	3239	3269									3254	2	21.3	0.65	15.0	0.46	0.92	12.710	5.87
010	RP	1822	1975									1899	2	108.0	5.69	76.4	4.02	8.05	12.710	51.14
011	RP	1454	1451									1452	2	2.2	0.15	1.5	0.11	0.21	12.710	1.34
012	RP	2890	2870									2880	2	14.2	0.49	10.1	0.35	0.70	12.710	4.44
013	RP	6665	6632									6648	2	23.7	0.36	16.7	0.25	0.50	12.710	3.20
014	RP	2929	2822									2875	2	76.0	2.64	53.7	1.87	3.74	12.710	23.76
015	RP	3017	2948									2982	2	49.1	1.65	34.7	1.16	2.33	12.710	14.79
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% ($k=t_{crit}$)
001	RP	722	733	650	704	599	763	771	815	841	849	744	10	80.7	10.84	25.5	3.43	6.86	2.262	7.76
002	RP	159	165									162	2	3.9	2.41	2.8	1.71	3.41	12.710	21.69
003	RP	166										166	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	887	887									887	2	0.3	0.03	0.2	0.02	0.05	12.710	0.31
009	RP	620	896									758	2	195.4	25.79	138.2	18.24	36.48	12.710	231.81
010	RP	331	522									427	2	135.1	31.67	95.5	22.39	44.78	12.710	284.59
011	RP	262	337									299	2	53.3	17.81	37.7	12.60	25.19	12.710	160.10
012	RP	514	649									581	2	95.6	16.44	67.6	11.62	23.25	12.710	147.75
013	RP	1934	1988									1961	2	37.9	1.93	26.8	1.37	2.73	12.710	17.36
014	RP	817	603									710	2	150.7	21.22	106.5	15.01	30.01	12.710	190.74
015	RP	761	672									717	2	62.7	8.75	44.3	6.19	12.37	12.710	78.62

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Ile Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	313	309	289	304	305	309	306	313	309	310	307	10	6.8	2.22	2.1	0.70	1.40	2.262	1.59
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	300	300									300	2	0.1	0.03	0.1	0.02	0.04	12.710	0.29
009	RP	337	361									349	2	17.0	4.86	12.0	3.44	6.88	12.710	43.71
010	RP	344	343									344	2	0.6	0.18	0.4	0.13	0.26	12.710	1.65
011	RP	267	287									277	2	13.8	4.99	9.8	3.53	7.06	12.710	44.88
012	RP	316	343									329	2	19.7	5.97	13.9	4.22	8.44	12.710	53.64
013	RP	200	200									200	2	0.1	0.07	0.1	0.05	0.09	12.710	0.59
014	RP	263	337									300	2	52.1	17.35	36.8	12.27	24.53	12.710	155.92
015	RP	290	341									316	2	36.0	11.41	25.5	8.07	16.13	12.710	102.53
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% ($k=t_{crit}$)
001	RP	42	42	35	38	31	39	38	40	39	39	38	10	3.3	8.55	1.0	2.70	5.41	2.262	6.12
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	47	47									47	2	0.2	0.37	0.1	0.26	0.52	12.710	3.33
009	RP	65	99									82	2	24.4	29.84	17.3	21.10	42.20	12.710	268.19
010	RP	63	91									77	2	20.0	26.03	14.1	18.41	36.82	12.710	233.98
011	RP	48	67									57	2	13.1	22.85	9.3	16.16	32.32	12.710	205.38
012	RP	56	78									67	2	15.2	22.78	10.8	16.11	32.22	12.710	204.74
013	RP	58	60									59	2	1.4	2.35	1.0	1.66	3.33	12.710	21.14
014	RP	73	72									73	2	0.9	1.30	0.7	0.92	1.84	12.710	11.72
015	RP	73	78									76	2	3.3	4.32	2.3	3.05	6.11	12.710	38.80

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Aile/Ile		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	0.135	0.137	0.121	0.125	0.103	0.127	0.124	0.128	0.127	0.125	0.125	10	0.0093	7.47	0.0030	2.36	4.72	2.262	5.34
002	RP	0.259	0.269									0.264	2	0.0068	2.58	0.0048	1.82	3.64	12.710	23.16
003	RP	0.255										0.255	1							
004	IE	0.136	0.133									0.135	2	0.0021	1.58	0.0015	1.12	2.23	12.710	14.17
005	IE	0.136	0.142									0.139	2	0.0042	3.05	0.0030	2.16	4.32	12.710	27.43
006[1]	GC_A	0.127										0.127	1							
007[1]	GC_A	0.159										0.159	10	0.0140	8.81	0.0044	2.78	5.57	2.262	6.30
008	RP	0.155	0.156									0.156	2	0.0007	0.45	0.0005	0.32	0.64	12.710	4.09
009	RP	0.191	0.274									0.233	2	0.0585	25.16	0.0414	17.79	35.58	12.710	226.13
010	RP	0.182	0.264									0.223	2	0.0585	26.21	0.0413	18.53	37.07	12.710	235.58
011	RP	0.180	0.232									0.206	2	0.0370	17.96	0.0262	12.70	25.40	12.710	161.42
012	RP	0.178	0.226									0.202	2	0.0342	16.93	0.0242	11.97	23.94	12.710	152.13
013	RP	0.290	0.300									0.295	2	0.0067	2.29	0.0048	1.62	3.23	12.710	20.55
014	RP	0.279	0.214									0.246	2	0.0459	18.63	0.0325	13.17	26.35	12.710	167.45
015	RP	0.252	0.228									0.240	2	0.0171	7.11	0.0121	5.03	10.05	12.710	63.88

[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**

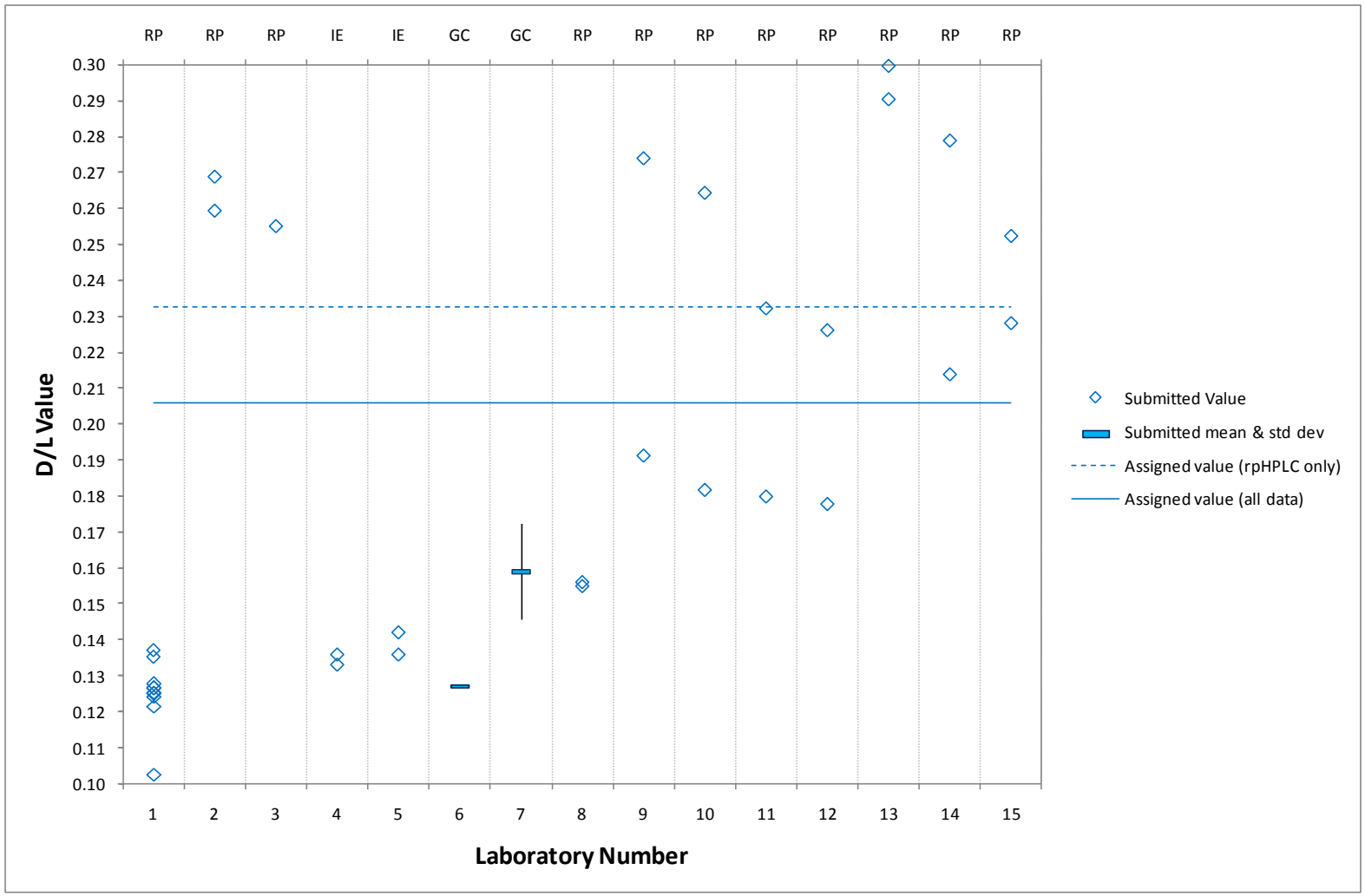


Figure 4.24: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **D-Alloisoleucine/L-Isoleucine** (value of n displayed).

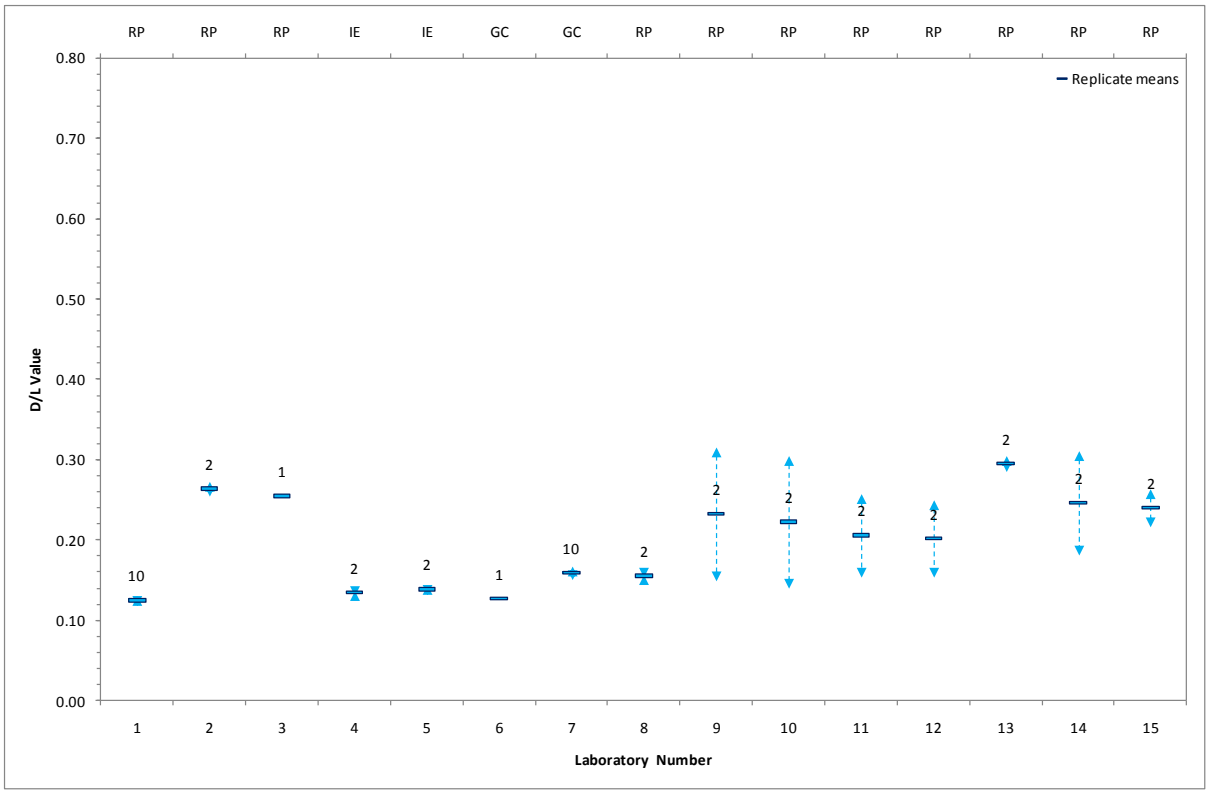


Figure 4.25: Experimental Expanded Uncertainty **(k=$t_{(0.05,df)}$)** of the Mean D/L value for **D-Alloisoleucine/L-Isoleucine** (value of n displayed).

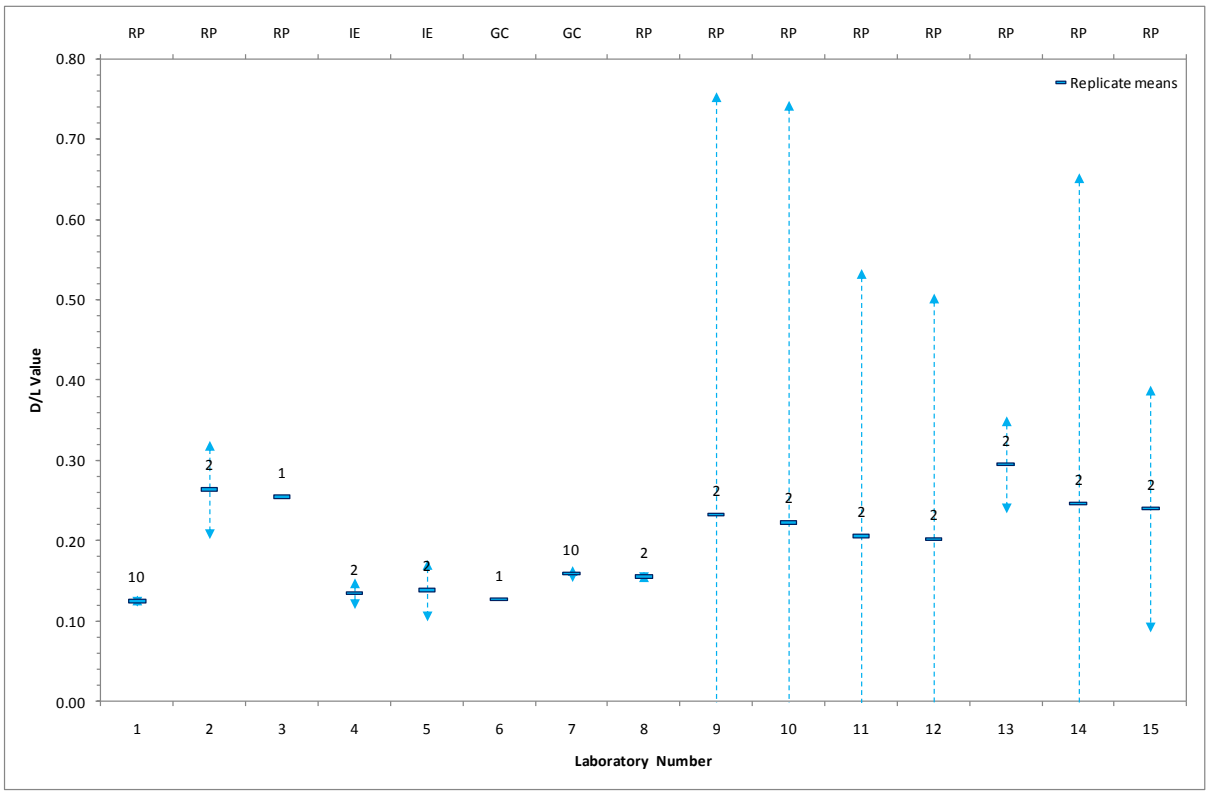


Table 4.26: Summary Statistics for L and D **Leucine** Peak Area Data

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)**	**Exp U% ($k=t_{crit}$)**
001	RP	10403	10566	10978	11156	11753	11941	12299	12294	13164	13399	11795	10	1027.0	8.71	324.8	2.75	5.51	2.262	6.23
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	12700	12643									12671	2	39.9	0.32	28.2	0.22	0.45	12.710	2.83
009	RP	6229	6257									6243	2	19.9	0.32	14.1	0.23	0.45	12.710	2.87
010	RP	3528	3766									3647	2	168.6	4.62	119.2	3.27	6.54	12.710	41.55
011	RP	2820	2854									2837	2	24.3	0.86	17.2	0.61	1.21	12.710	7.69
012	RP	5452	5487									5469	2	24.7	0.45	17.4	0.32	0.64	12.710	4.05
013	RP	13484	13443									13464	2	29.4	0.22	20.8	0.15	0.31	12.710	1.96
014	RP	5685	5269									5477	2	294.1	5.37	208.0	3.80	7.59	12.710	48.26
015	RP	5810	5733									5771	2	54.2	0.94	38.3	0.66	1.33	12.710	8.44
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% ($k=t_{crit}$)**
001	RP	3065	3252	2874	3148	3635	3635	4036	4100	4488	4719	3695	10	626.4	16.95	198.1	5.36	10.72	2.262	12.13
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	3050	2914									2982	2	96.0	3.22	67.9	2.28	4.55	12.710	28.93
009	RP	1841	1715									1778	2	89.3	5.02	63.1	3.55	7.10	12.710	45.13
010	RP	1022	1043									1033	2	14.5	1.40	10.2	0.99	1.98	12.710	12.60
011	RP	551	612									582	2	42.7	7.34	30.2	5.19	10.38	12.710	65.97
012	RP	1607	1584									1595	2	16.6	1.04	11.8	0.74	1.47	12.710	9.36
013	RP																			
014	RP	1945	1553									1749	2	277.3	15.85	196.0	11.21	22.42	12.710	142.48
015	RP	1719	1606									1663	2	80.1	4.82	56.6	3.41	6.81	12.710	43.30
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	4.224	4.239									4.232	2	0.0106	0.25	0.0075	0.18	0.35	12.710	2.25
005	IE	3.259	3.781									3.520	2	0.3691	10.49	0.2610	7.41	14.83	12.710	94.24

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Leu Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP	611	610	593	603	613	613	605	603	612	612	607	10	6.3	1.04	2.0	0.33	0.66	2.262	0.75
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	667	667									667	2	0.6	0.09	0.4	0.06	0.13	12.710	0.81
009	RP	870	927									898	2	40.7	4.53	28.8	3.20	6.41	12.710	40.71
010	RP	893	877									885	2	11.1	1.25	7.8	0.89	1.77	12.710	11.25
011	RP	694	756									725	2	43.5	6.00	30.7	4.24	8.48	12.710	53.90
012	RP	798	880									839	2	58.0	6.91	41.0	4.89	9.77	12.710	62.12
013	RP	541	543									542	2	1.1	0.20	0.8	0.14	0.29	12.710	1.82
014	RP	685	844									765	2	112.1	14.65	79.2	10.36	20.73	12.710	131.71
015	RP	749	889									819	2	99.2	12.11	70.2	8.56	17.13	12.710	108.83
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% ($k=t_{crit}$)
001	RP	180	188	155	170	190	187	199	201	209	215	189	10	17.9	9.48	5.7	3.00	6.00	2.262	6.78
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP	160	154									157	2	4.4	2.81	3.1	1.99	3.98	12.710	25.29
009	RP	257	254									256	2	2.1	0.81	1.5	0.57	1.15	12.710	7.30
010	RP	259	243									251	2	11.2	4.47	7.9	3.16	6.33	12.710	40.20
011	RP	136	162									149	2	18.5	12.46	13.1	8.81	17.62	12.710	111.98
012	RP	235	254									245	2	13.3	5.42	9.4	3.83	7.67	12.710	48.73
013	RP																			
014	RP	235	249									242	2	10.0	4.16	7.1	2.94	5.88	12.710	37.37
015	RP	222	249									235	2	19.4	8.25	13.7	5.83	11.67	12.710	74.14

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
D/L Leu		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
001	RP	0.295	0.308	0.262	0.282	0.309	0.304	0.328	0.334	0.341	0.352	0.311	10	0.0278	8.93	0.0088	2.83	5.65	2.262	6.39
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006[1]	GC_A	0.216										0.216	1							
007[1]	GC_A	0.207										0.207	10	0.0060	2.90	0.0019	0.92	1.83	2.262	2.07
008	RP	0.240	0.231									0.236	2	0.0064	2.70	0.0045	1.91	3.82	12.710	24.29
009	RP	0.296	0.274									0.285	2	0.0152	5.34	0.0108	3.78	7.55	12.710	48.00
010	RP	0.290	0.277									0.283	2	0.0091	3.22	0.0065	2.28	4.56	12.710	28.96
011	RP	0.196	0.214									0.205	2	0.0133	6.49	0.0094	4.59	9.17	12.710	58.29
012	RP	0.295	0.289									0.292	2	0.0044	1.49	0.0031	1.06	2.11	12.710	13.41
013	RP																			
014	RP	0.342	0.295									0.318	2	0.0335	10.53	0.0237	7.45	14.89	12.710	94.63
015	RP	0.296	0.280									0.288	2	0.0112	3.88	0.0079	2.74	5.49	12.710	34.87

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

GC_A= derived using peak area

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

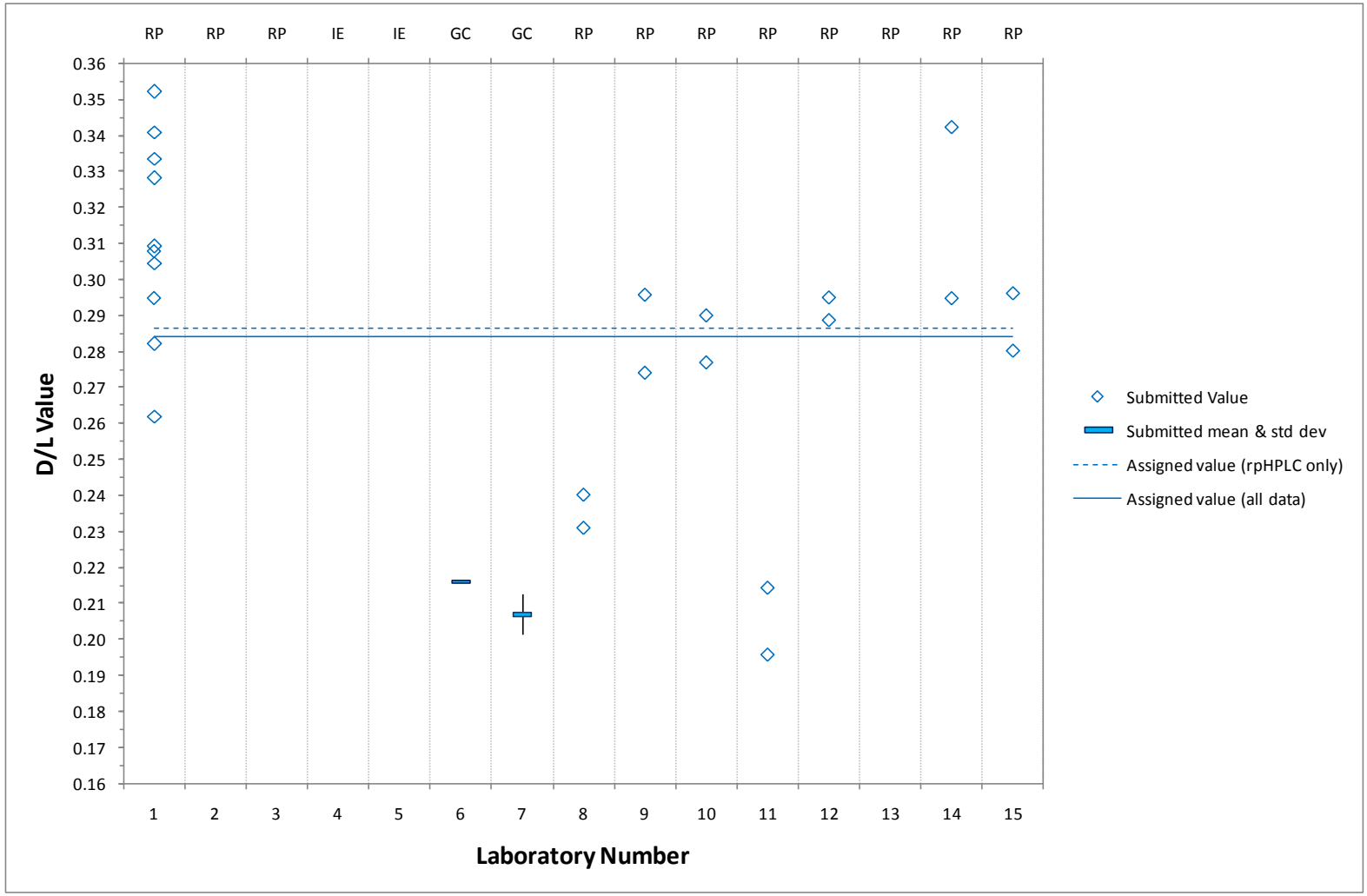


Figure 4.27: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Leucine** (value of n displayed).

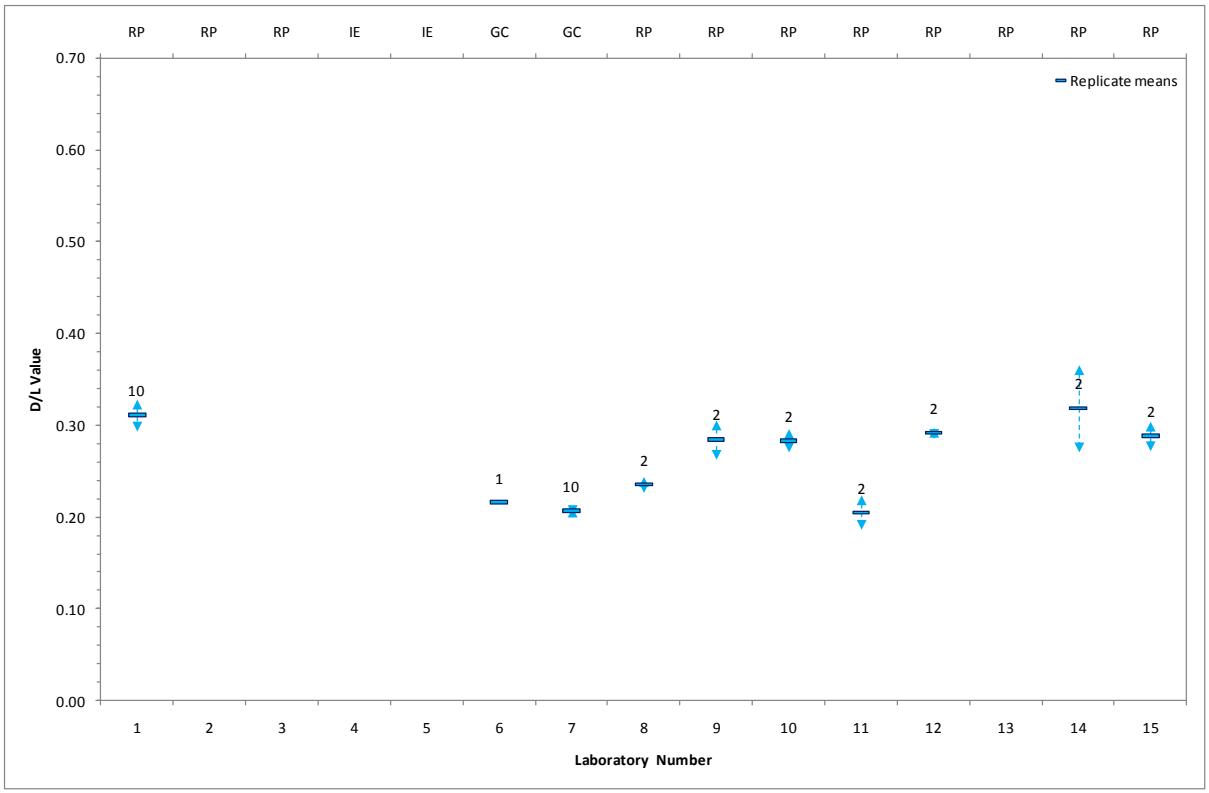


Figure 4.28: Experimental Expanded Uncertainty **($k=t_{(0.05,df)}$)** of the Mean D/L value for **Leucine** (value of n displayed).

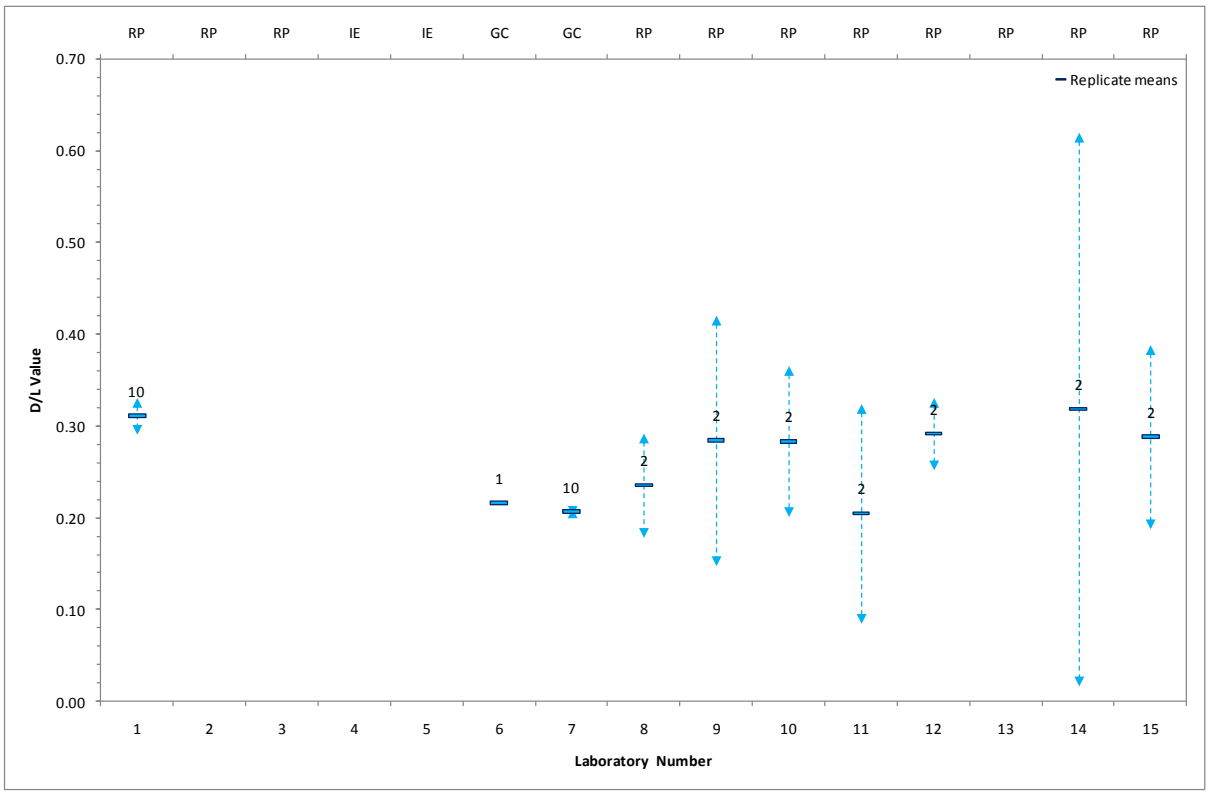


Table 4.29: Summary Statistics for L and D **Tyrosine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Tyr peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% ($k=t_{crit}$)**
001	RP																			
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	1607	1638									1622	2	22.4	1.38	15.8	0.98	1.95	12.710	12.40
010	RP	998	1064									1031	2	46.9	4.55	33.2	3.22	6.43	12.710	40.88
011	RP	771	753									762	2	12.2	1.61	8.7	1.14	2.27	12.710	14.43
012	RP	928	830									879	2	69.1	7.86	48.8	5.56	11.12	12.710	70.65
013	RP																			
014	RP	1053	992									1023	2	43.5	4.25	30.7	3.00	6.01	12.710	38.19
015	RP	1281	1209									1245	2	50.8	4.08	35.9	2.88	5.77	12.710	36.66
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% ($k=t_{crit}$)**
001	RP																			
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	450	438									444	2	8.3	1.86	5.8	1.31	2.63	12.710	16.71
010	RP	284	287									285	2	2.8	0.98	2.0	0.69	1.39	12.710	8.81
011	RP	217	211									214	2	4.0	1.86	2.8	1.32	2.63	12.710	16.72
012	RP	264	237									251	2	19.5	7.79	13.8	5.51	11.02	12.710	70.01
013	RP																			
014	RP																			
015	RP	322	298									310	2	16.6	5.35	11.7	3.78	7.56	12.710	48.05

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Tyr Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP																			
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	145	157									151	2	8.4	5.59	6.0	3.95	7.90	12.710	50.22
010	RP	163	160									161	2	2.1	1.33	1.5	0.94	1.88	12.710	11.92
011	RP	122	129									125	2	4.4	3.54	3.1	2.50	5.00	12.710	31.80
012	RP	88	86									87	2	1.2	1.40	0.9	0.99	1.98	12.710	12.60
013	RP																			
014	RP	82	102									92	2	14.5	15.76	10.3	11.15	22.29	12.710	141.68
015	RP	107	121									114	2	10.2	8.99	7.2	6.35	12.71	12.710	80.76
D-Tyr Conc		a	b	c	d	e	f	g	h	i	j	mean	n	std dev	CV%	std u	RSU%	Exp U% (k=2)	t critical (0.05,df)	Exp U% ($k=t_{crit}$)
001	RP																			
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	41	42									41	2	1.0	2.35	0.7	1.66	3.33	12.710	21.14
010	RP	46	43									45	2	2.2	4.89	1.5	3.46	6.92	12.710	43.99
011	RP	34	36									35	2	1.2	3.28	0.8	2.32	4.64	12.710	29.51
012	RP	25	24									25	2	0.3	1.33	0.2	0.94	1.88	12.710	11.96
013	RP																			
014	RP																			
015	RP	27	30									28	2	2.2	7.72	1.5	5.46	10.92	12.710	69.40

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% ($k=t_{crit}$)
001	RP																			
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	0.280	0.268									0.274	2	0.0089	3.24	0.0063	2.29	4.58	12.710	29.10
010	RP	0.284	0.270									0.277	2	0.0099	3.57	0.0070	2.52	5.05	12.710	32.08
011	RP	0.281	0.280									0.281	2	0.0007	0.25	0.0005	0.18	0.36	12.710	2.29
012	RP	0.285	0.285									0.285	2	0.0002	0.07	0.0001	0.05	0.10	12.710	0.64
013	RP																			
014	RP																			
015	RP	0.251	0.247									0.249	2	0.0032	1.27	0.0022	0.90	1.79	12.710	11.40

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

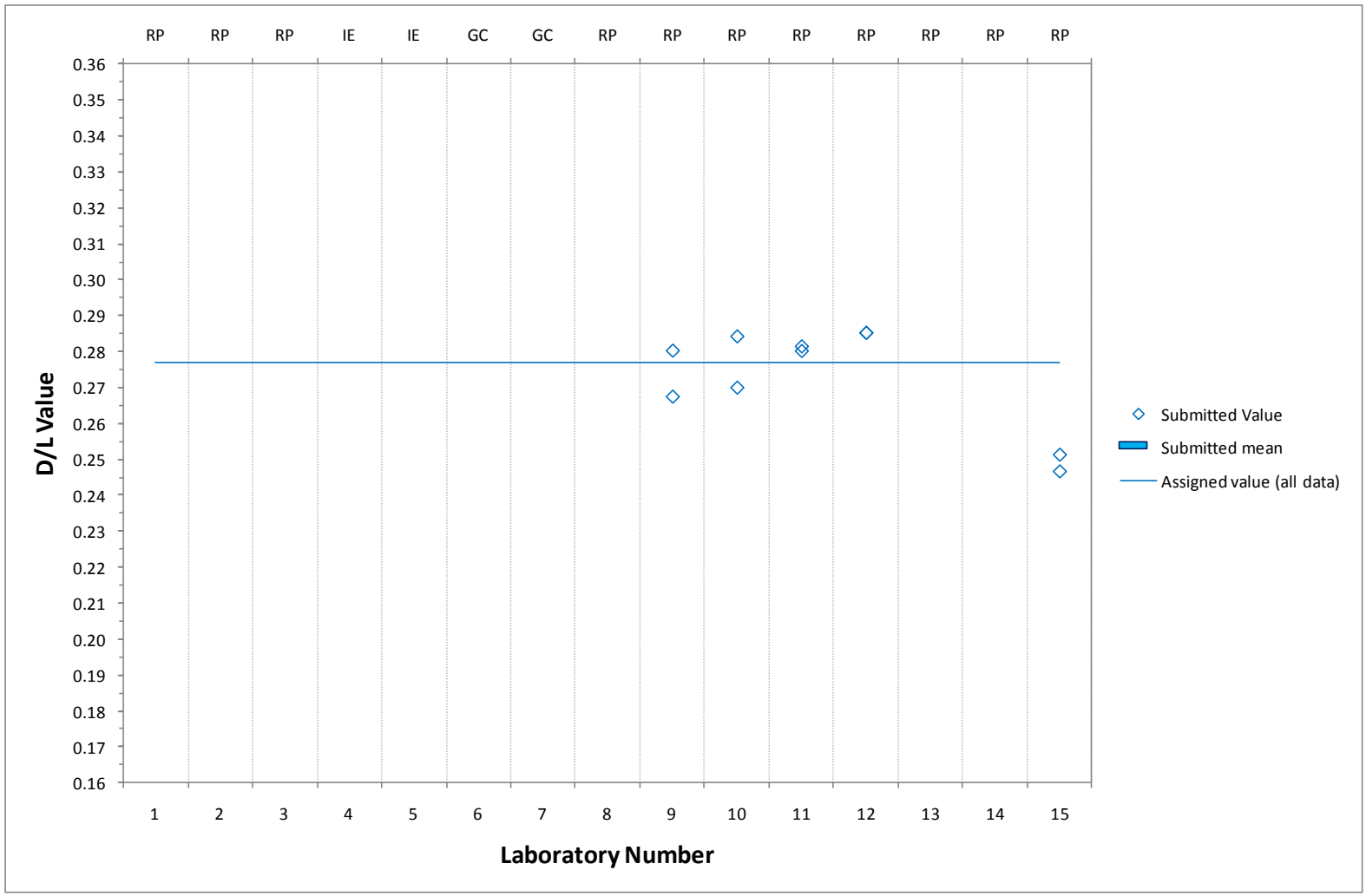


Figure 4.30: Experimental Expanded Uncertainty **(k=2)** of the Mean D/L value for **Tyrosine** (value of n displayed).

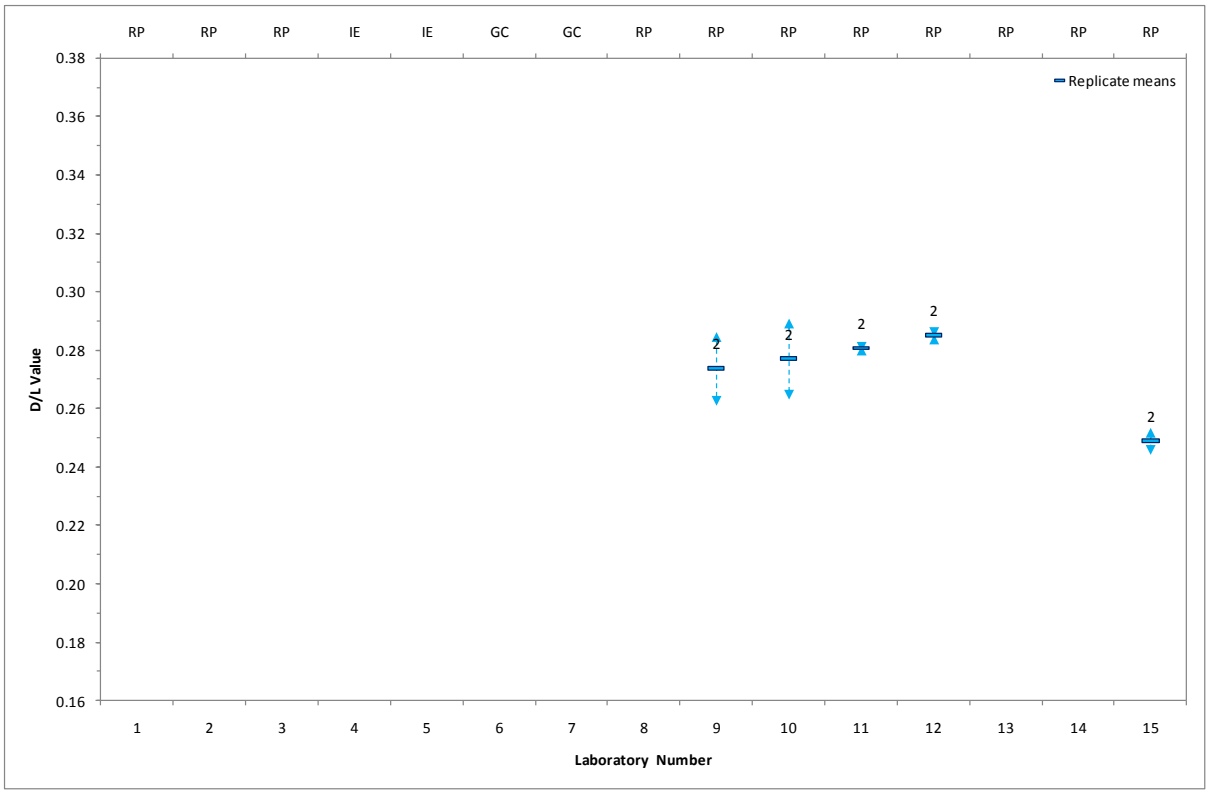


Figure 4.31: Experimental Expanded Uncertainty **(k=$t_{(0.05,df)}$)** of the Mean D/L value for **Tyrosine** (value of n displayed).

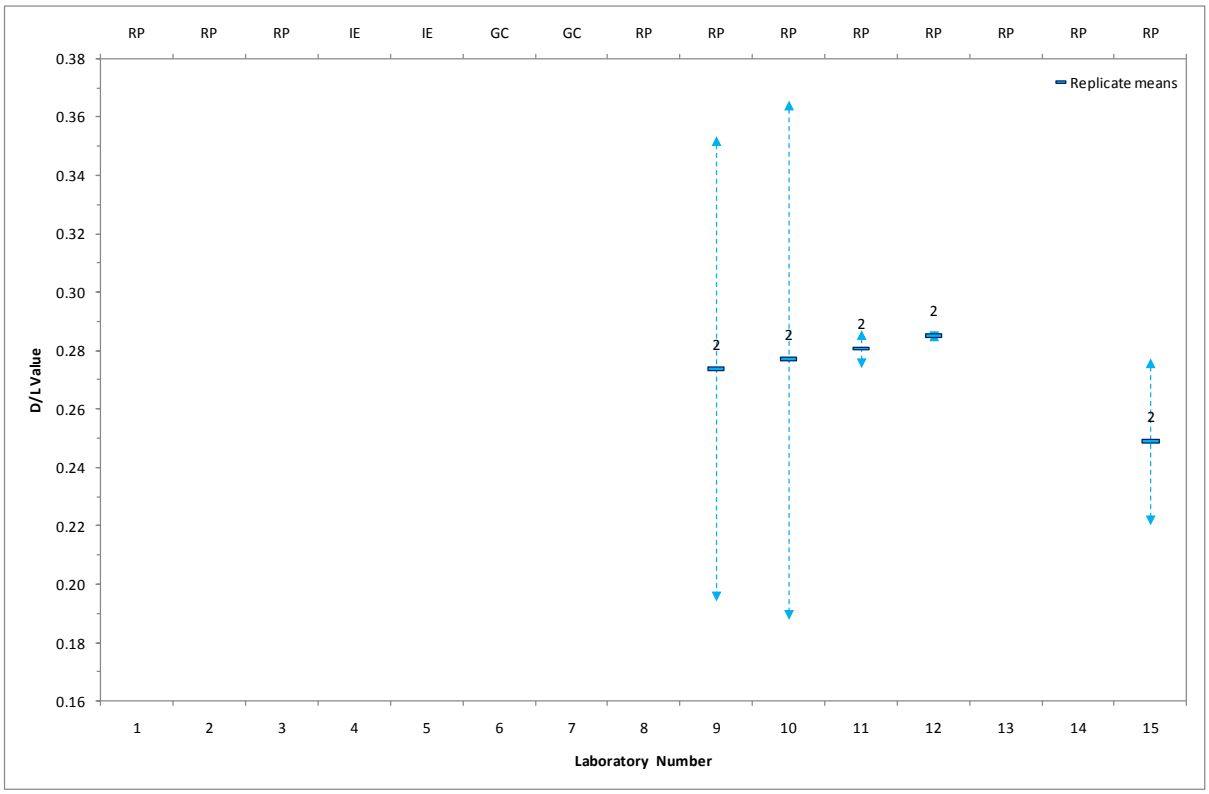


Table 4.32: Summary Statistics for L and D **Methionine** Peak Area Data

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-Met peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% (k=t_{crit})**
001	RP																			
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	428	340									384	2	62.3	16.23	44.1	11.48	560.1	428	340
010	RP	183	206									194	2	16.8	8.66	11.9	6.12	151.3	183	206
011	RP																			
012	RP	489	400									445	2	62.8	14.11	44.4	9.98	564.1	489	400
013	RP	3230	3215									3223	2	10.5	0.33	7.5	0.23	94.7	3230	3215
014	RP	1355	1295									1325	2	42.5	3.21	30.0	2.27	381.9	1355	1295
015	RP	330	275									303	2	39.1	12.91	27.6	9.13	351.2	330	275
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})**
001	RP																			
002	RP																			
003	RP																			
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	121	66									94	2	39.2	41.82	27.7	29.57	352.4	121	66
010	RP	69	93									81	2	17.1	20.97	12.1	14.82	153.4	69	93
011	RP																			
012	RP																			
013	RP																			
014	RP	689	613									651	2	53.7	8.26	38.0	5.84	483.0	689	613
015	RP	163	138									150	2	17.9	11.91	12.7	8.42	160.9	163	138

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

Lab No	method	Submitted Replicate data										Standard Deviation				Uncertainty of Mean & Expanded U at 95% CL				
L-homoArginine peak area		**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**mean**	**n**	**std dev**	**CV%**	**std u**	**RSU%**	**Exp U% (k=2)**	**t critical (0.05,df)**	**Exp U% (k=t_{crit})**
001	RP	5110	5199	5550	5552	5747	5844	6099	6115	6457	6570	5824	10	491.5	8.44	155.4	2.67	5.34	2.262	6.04
002	RP	461	468									465	2	4.6	0.99	3.2	0.70	1.39	12.710	8.86
003	RP	353										353	1							
004	IE																			
005	IE																			
006	GC																			
007	GC																			
008	RP																			
009	RP	1646	1551									1599	2	67.3	4.21	47.6	2.98	5.95	12.710	37.84
010	RP	908	986									947	2	55.6	5.87	39.3	4.15	8.31	12.710	52.79
011	RP	934	868									901	2	46.3	5.14	32.8	3.64	7.27	12.710	46.22
012	RP	1570	1433									1501	2	97.0	6.46	68.6	4.57	9.14	12.710	58.08
013	RP	2862	2845									2854	2	12.0	0.42	8.5	0.30	0.60	12.710	3.78
014	RP	958	721									839	2	167.4	19.95	118.4	14.10	28.21	12.710	179.26
015	RP	891	741									816	2	106.4	13.04	75.2	9.22	18.44	12.710	117.20
Norleucine peak height		**a**	**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)**
001	RP																			
002	RP																			
003	RP																			
004	IE	0.521	0.499											0.510	2	0.0156	3.05	0.0110	2.16	4.31	12.710	27.41
005	IE	0.416	0.461											0.439	2	0.0318	7.26	0.0225	5.13	10.26	12.710	65.22
006	GC																			
007	GC																			
008	RP																			
009	RP																			
010	RP																			
011	RP																			
012	RP																			
013	RP																			
014	RP																			
015	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 the absence of independently determined performance criteria, it was decided to present the data as an assessment of relative bias (%), such that;

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

Satisfactory performance was assessed as plus or minus twice the standard deviation of the assigned value, representing 95% confidence limits, i.e.; ± 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 Opercula Test Material

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

Whilst assessing the data, in many cases it became clear that the robust mean (Ellison, 2002b, RSC Analytical Methods Committee, 1989, RSC Analytical Methods Committee, 2001) was strongly influenced by extreme values resulting in a skewed distribution with a high or low end tail. This appeared largely influenced by method and on occasions by an individual laboratory where more than one result was submitted using the same method, but carried out using a different instrument or analyst. In addition, when determining the mode (Ellison, 2002a, RSC Analytical Methods Committee, 2006, Lowthian and Thompson, 2002), it became clear that due to the low numbers of results, additional modes were identified due to only a couple of values and in some cases only a single data point. Plots showing the modal distributions derived using the kernel density Excel add-in (Ellison, 2002a) are shown against each histogram for amino acids with eight or more data points.

In cases where there were two evenly matched modes or where a smaller second mode was predominated by data using a specific method such as GC, it would not be appropriate to penalise

these laboratories by comparison against an assigned value determined from the primary or first mode. There is no judgment being made as to which set of results is '*correct*', therefore, it would not be appropriate to calculate performance for GC results using an assigned value determined from HPLC values if the GC data clustered differently. In situations such as this where the method may be empirical, the mode should not be used. Regrettably submitted results by GC were limited making it difficult to know whether the observed differences are genuine method differences or simply extreme values.

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

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

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

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

5.5 Interpreting Results - a word of caution.

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

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

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

Table 5.1: Results and Relative Percentage Bias for Total Hydrolysed Amino Acids in Opercula 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	0.573	assigned value	0.572	assigned value	0.165	assigned value	0.164
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
001	RP	0.549	-4.1	0.549	-4.0	0.150	-9.3	0.150	-9.0
002	RP	0.552	-3.6	0.552	-3.4	0.140	-15.0	0.140	-14.6
003	RP	0.571	-0.3	0.571	-0.1	0.144	-12.7	0.144	-12.3
004	IE								
005	IE								
006	GC	0.650	13.5			0.202	22.5		
007	GC	0.631	10.2			0.174	5.5		
008	RP	0.576	0.5	0.576	0.7	0.163	-1.2	0.163	-0.8
009	RP	0.576	0.7	0.576	0.8	0.166	0.4	0.166	0.8
010	RP	0.571	-0.3	0.571	-0.1	0.165	0.0	0.165	0.4
011	RP	0.580	1.3	0.580	1.5	0.166	0.5	0.166	0.9
012	RP	0.577	0.9	0.577	1.0	0.167	1.0	0.167	1.4
013	RP	0.573	0.0	0.573	0.1	0.166	0.6	0.166	1.0
014	RP	0.571	-0.3	0.571	-0.1	0.164	-0.4	0.164	0.0
015	RP	0.570	-0.4	0.570	-0.3	0.164	-0.8	0.164	-0.4

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 Opercula 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	0.662	assigned value	0.803	assigned value	0.263	assigned value	0.264
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
001	RP	0.647	-2.2			0.279	5.8	0.279	5.5
002	RP	0.662	0.0	0.979	21.9	0.273	3.5	0.273	3.1
003	RP	0.667	0.8	0.921	14.7	0.286	8.6	0.286	8.2
004	IE								
005	IE								
006	GC					0.246	-6.6		
007	GC					0.265	0.6		
008	RP	0.673	1.7			0.271	2.7	0.271	2.3
009	RP	0.663	0.2	0.860	7.1	0.265	0.4	0.265	0.1
010	RP	0.644	-2.7	0.796	-0.9	0.255	-3.2	0.255	-3.5
011	RP	0.653	-1.3	0.668	-16.9	0.262	-0.4	0.262	-0.7
012	RP	0.667	0.8	0.713	-11.2	0.263	0.0	0.263	-0.3
013	RP	0.668	0.9	0.366	-54.4	0.254	-3.6	0.254	-3.9
014	RP	0.655	-1.0	0.948	18.0	0.251	-4.9	0.251	-5.2
015	RP	0.655	-1.0	0.803	0.0	0.253	-4.1	0.253	-4.4

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 Opercula 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	0.137	assigned value	0.137	assigned value	0.304	assigned value	0.305
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
001	RP	0.139	1.1	0.139	1.1	0.298	-1.9	0.298	-2.3
002	RP	0.141	2.9	0.141	2.9	0.299	-1.7	0.141	-2.0
003	RP	0.144	5.1	0.144	5.1	0.326	7.2	0.144	6.8
004	IE								
005	IE								
006	GC	0.137	0.0			0.297	-2.3		
007	GC	0.109	-20.4			0.280	-7.9		
008	RP	0.137	0.0	0.137	0.0	0.344	13.0	0.344	12.6
009	RP	0.131	-4.3	0.131	-4.3	0.308	1.3	0.308	0.9
010	RP	0.122	-10.7	0.122	-10.7	0.300	-1.2	0.300	-1.6
011	RP	0.122	-10.7	0.122	-10.7	0.305	0.4	0.305	0.0
012	RP	0.128	-6.3	0.128	-6.3	0.304	0.0	0.304	-0.4
013	RP	0.141	3.1	0.141	3.1	0.314	3.4	0.314	3.0
014	RP	0.149	8.4	0.149	8.4	0.309	1.5	0.309	1.1
015	RP	0.126	-8.0	0.126	-8.0	0.297	-2.3	0.297	-2.6

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

Table 5.1: Results and Relative Percentage Bias for Total Hydrolysed Amino Acids in Opercula 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	0.206	assigned value	0.233	assigned value	0.284	assigned value	0.286
		result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %	result D/L	relative bias %
001	RP	0.125	-39.3	0.125	-46.2	0.311	9.7	0.311	8.8
002	RP	0.264	28.2	0.264	13.5				
003	RP	0.255	23.8	0.255	9.6				
004	IE	0.135	-34.7						
005	IE	0.139	-32.5						
006	GC	0.127	-38.4			0.216	-24.0		
007	GC	0.159	-22.8			0.207	-27.1		
008	RP	0.156	-24.5	0.156	-33.2	0.236	-17.1	0.236	-17.8
009	RP	0.233	12.9	0.233	0.0	0.285	0.3	0.285	-0.6
010	RP	0.223	8.3	0.223	-4.1	0.283	-0.3	0.283	-1.1
011	RP	0.206	0.0	0.206	-11.4	0.205	-27.8	0.205	-28.4
012	RP	0.202	-2.0	0.202	-13.2	0.292	2.7	0.292	1.8
013	RP	0.295	43.2	0.295	26.8				
014	RP	0.246	19.5	0.246	5.9	0.318	12.1	0.318	11.2
015	RP	0.240	16.6	0.240	3.2	0.288	1.4	0.288	0.6

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

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

Lab No.	method	Total Hydrolysed Amino Acid (THAA)	
		Tyr D/L (rpHPLC)	
		assigned value	0.277
		result D/L	relative bias %
1	RP		
2	RP		
3	RP		
4	IE		
5	IE		
6	GC		
7	GC		
8	RP		
9	RP	0.274	-1.2
10	RP	0.277	0.0
11	RP	0.281	1.3
12	RP	0.285	2.9
13	RP		
14	RP		
15	RP	0.249	-10.1

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 5.2: 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 (alla)	13	0.573	0.0058	1.02	0.0016	0.28
Asx D/L (rpHPLC)	11	0.572	0.0067	1.17	0.0020	0.35
Glx D/L (all[a])	13	0.165	0.0024	1.47	0.0007	0.41
Glx D/L (rpHPLC)	11	0.164	0.0021	1.29	0.0006	0.39
Ser D/L (rpHPLC)	11	0.662	0.0093	1.41	0.0028	0.43
Arg D/L (rpHPLC)	9	0.803	0.1747	21.76	0.0582	7.25
Ala D/L (all[a])	13	0.263	0.0136	5.15	0.0038	1.43
Ala D/L (rpHPLC)	11	0.264	0.0136	5.14	0.0041	1.55
Val D/L (all[a])	13	0.137	0.0096	6.99	0.0027	1.94
Val D/L (rpHPLC)	11	0.137	0.0104	7.58	0.0031	2.28
Phe D/L (all[a])	13	0.304	0.0087	2.87	0.0024	0.79
Phe D/L (rpHPLC)	11	0.305	0.0092	3.01	0.0028	0.91
D-Aile/L-Ile (all[b])	15	0.206	0.0726	35.21	0.0187	9.09
D-Aile/L-Ile (rpHPLC)	11	0.233	0.0394	16.94	0.0119	5.11
Leu D/L (all[a])	10	0.284	0.0458	16.12	0.0145	5.10
Leu D/L (rpHPLC)	8	0.286	0.0225	7.86	0.0080	2.78
Tyr D/L (rpHPLC)	5	0.277	0.0055	1.99	0.0025	0.89

[a] = rpHPLC and GC data [b] = rpHPLC, GC and HPLC-IE data
m = number of replicate mean values sMAD = median absolute deviation
CV% = coefficient of variation 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 (alla)	0.573	9	13	69%
Asx D/L (rpHPLC)	0.572	9	11	82%
Glx D/L (all[a])	0.165	8	13	62%
Glx D/L (rpHPLC)	0.164	8	11	73%
Ser D/L (rpHPLC)	0.662	11	11	100%
Arg D/L (rpHPLC)	0.803	8	9	89%
Ala D/L (all[a])	0.263	13	13	100%
Ala D/L (rpHPLC)	0.264	11	11	100%
Val D/L (all[a])	0.137	12	13	92%
Val D/L (rpHPLC)	0.137	11	11	100%
Phe D/L (all[a])	0.304	10	13	77%
Phe D/L (rpHPLC)	0.305	9	11	100%
D-Aile/L-Ile (all[b])	0.206	15	15	100%
D-Aile/L-Ile (rpHPLC)	0.233	10	11	91%
Leu D/L (all[a])	0.284	10	10	100%
Leu D/L (rpHPLC)	0.286	6	8	75%
Tyr D/L (rpHPLC)	0.277	4	5	80%

[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

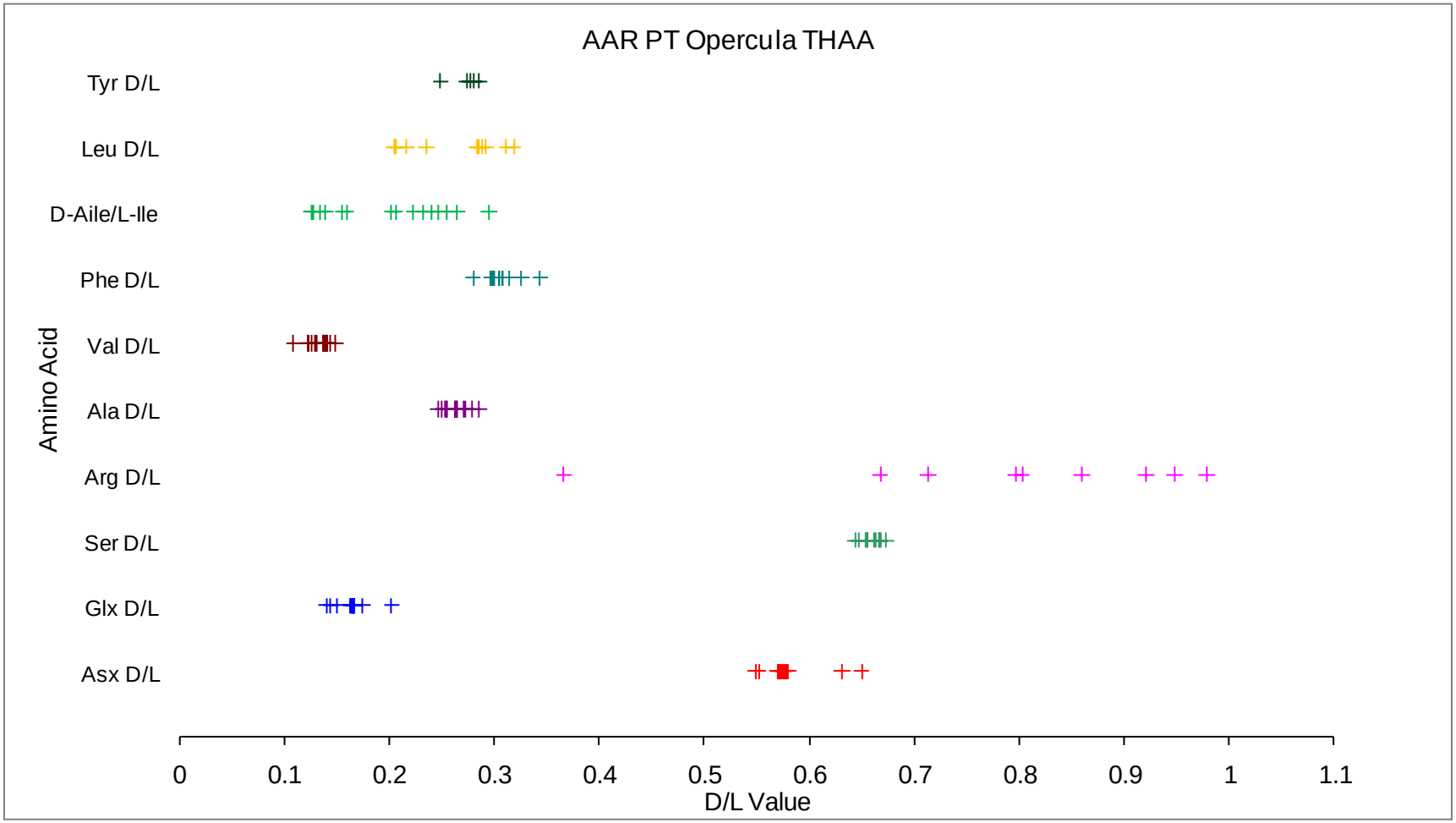


Figure 5.2: Relative Percentage Bias for **Aspartic Acid / Asparagine D/L Results (all data)** in Opercula Test Material

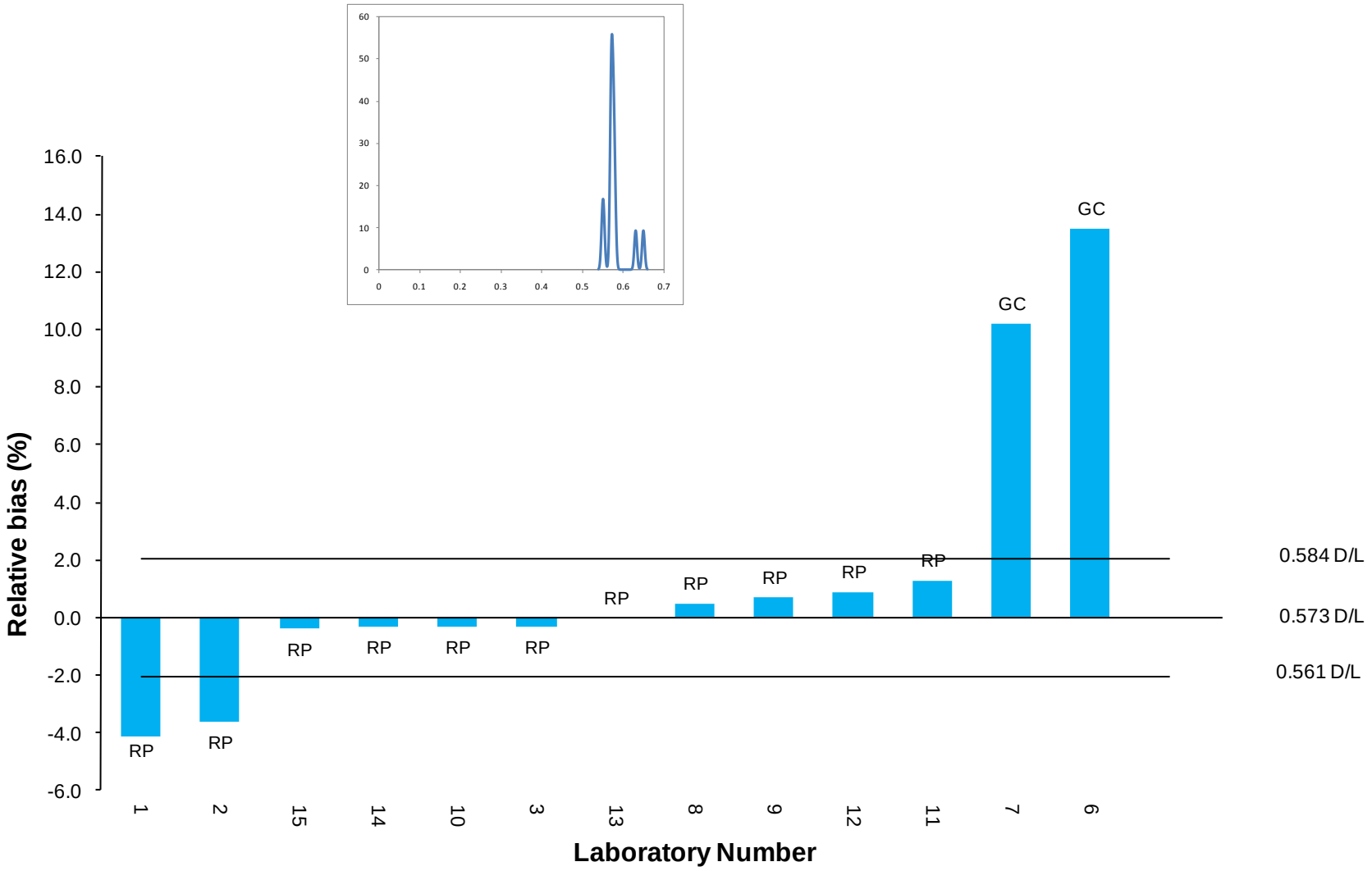


Figure 5.3: Relative Percentage Bias for **Aspartic Acid / Asparagine D/L Results (rpHPLC data only)** in Opercula Test Material

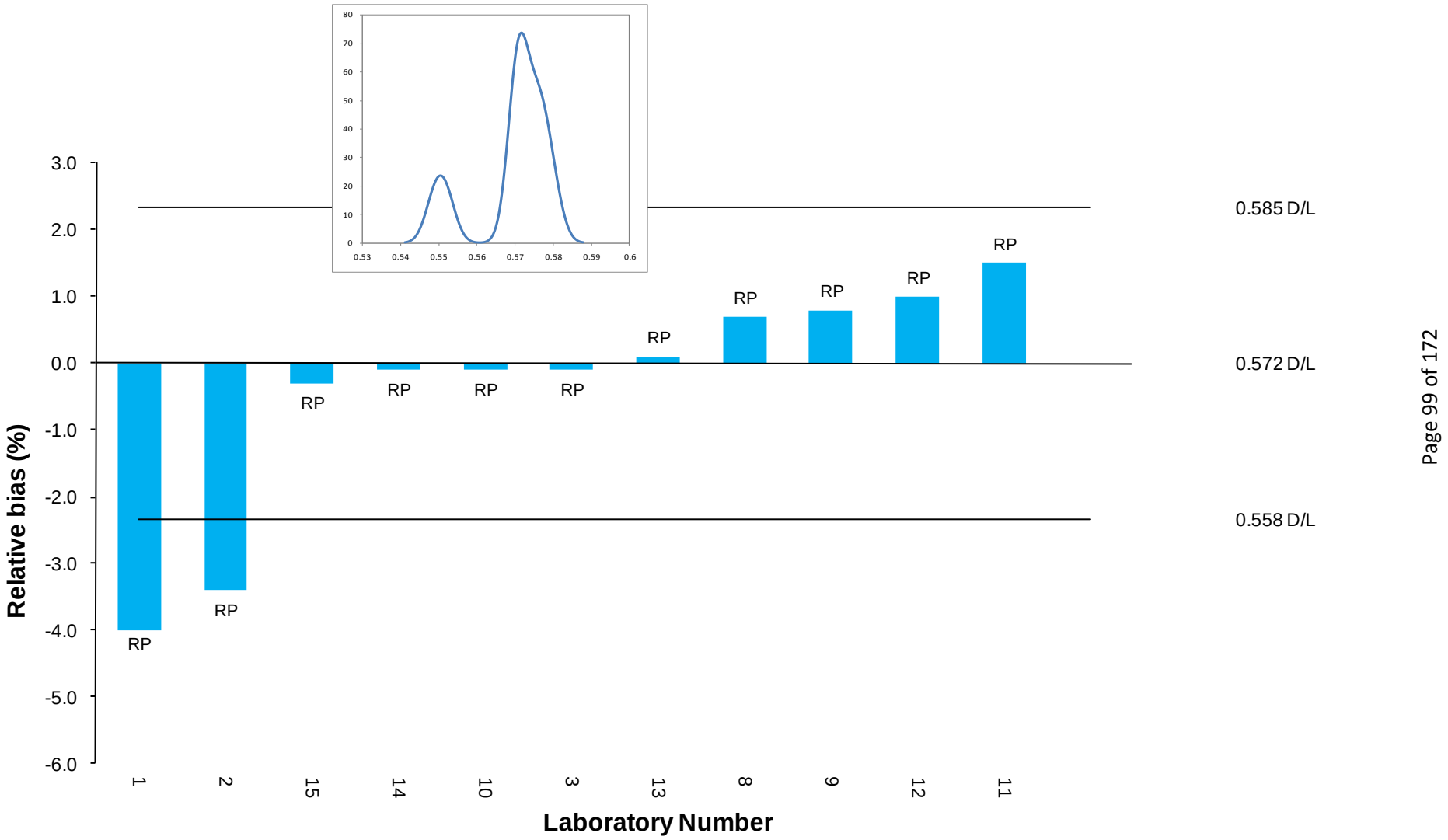


Figure 5.4: Relative Percentage Bias for **Glutamic Acid / Glutamate D/L Results (all data)** in Opercula Test Material

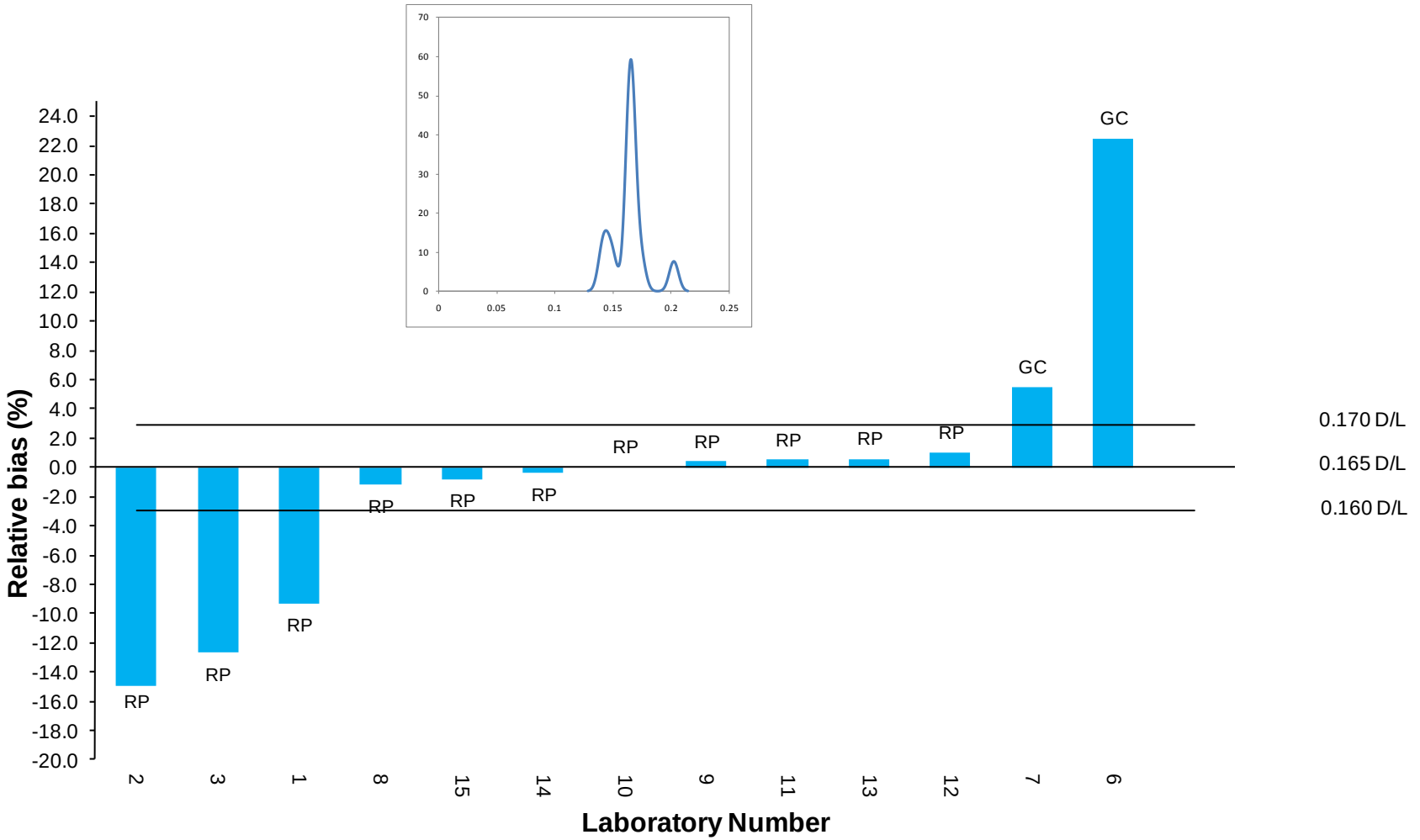


Figure 5.5: Relative Percentage Bias for **Glutamic Acid / Glutamate D/L Results (rpHPLC data only)** in Opercula Test Material

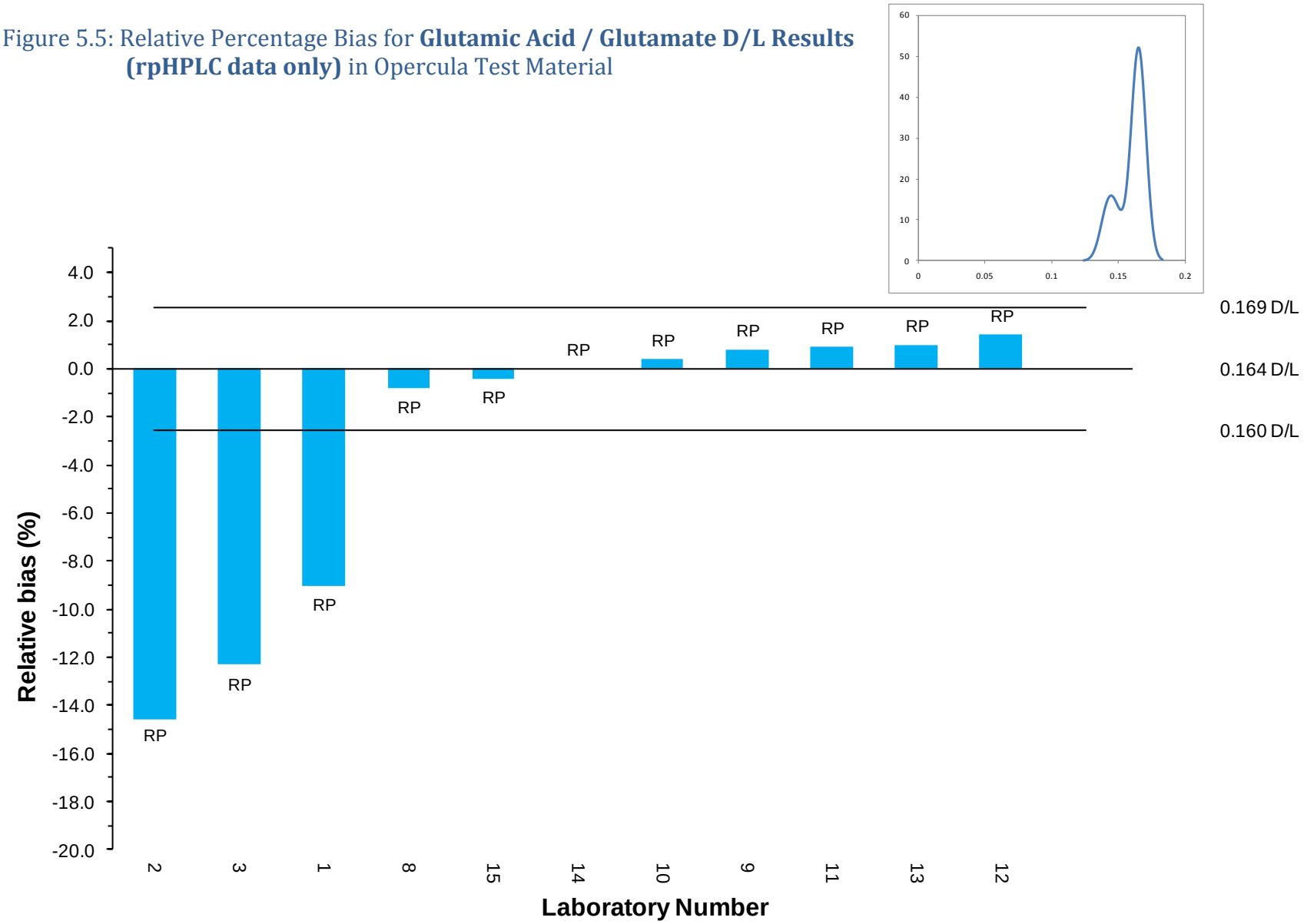


Figure 5.6: Relative Percentage Bias for **Serine D/L Results (all / rpHPLC data)** in Opercula Test Material

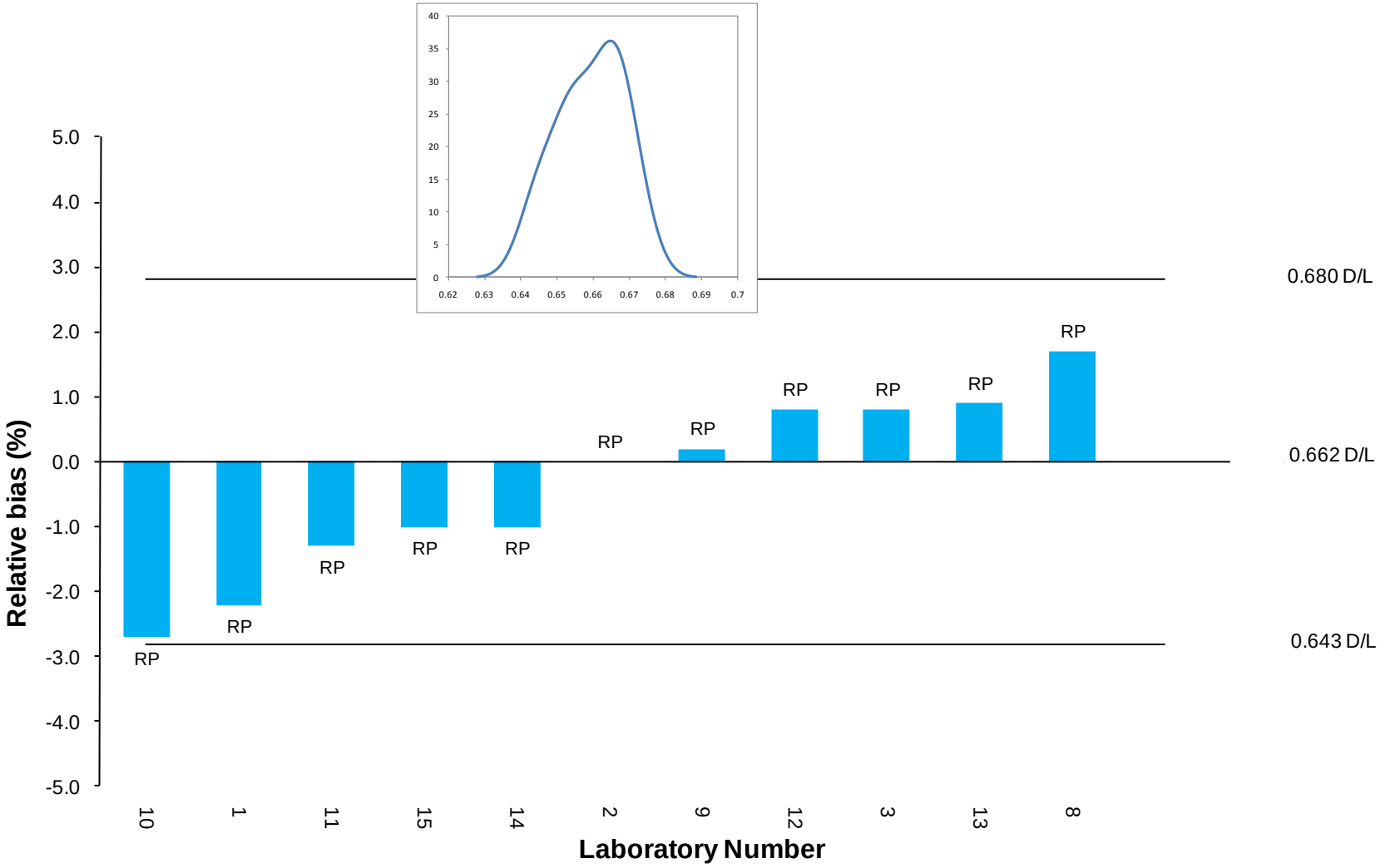


Figure 5.7: Relative Percentage Bias for **Arginine D/L Results (rpHPLC data only)** in Opercula Test Material

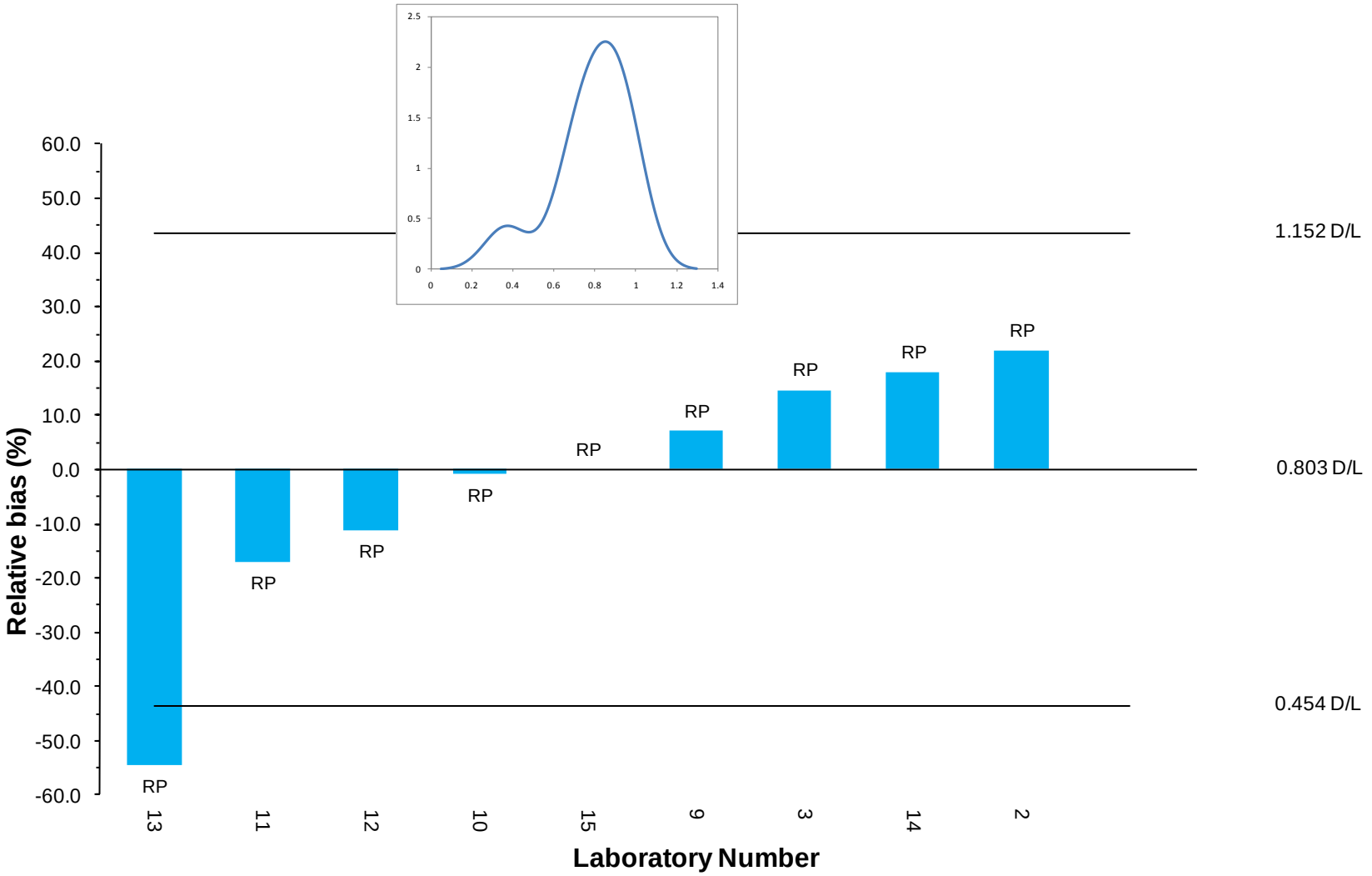


Figure 5.8: Relative Percentage Bias for **Alanine D/L Results (all data)** in Opercula Test Material

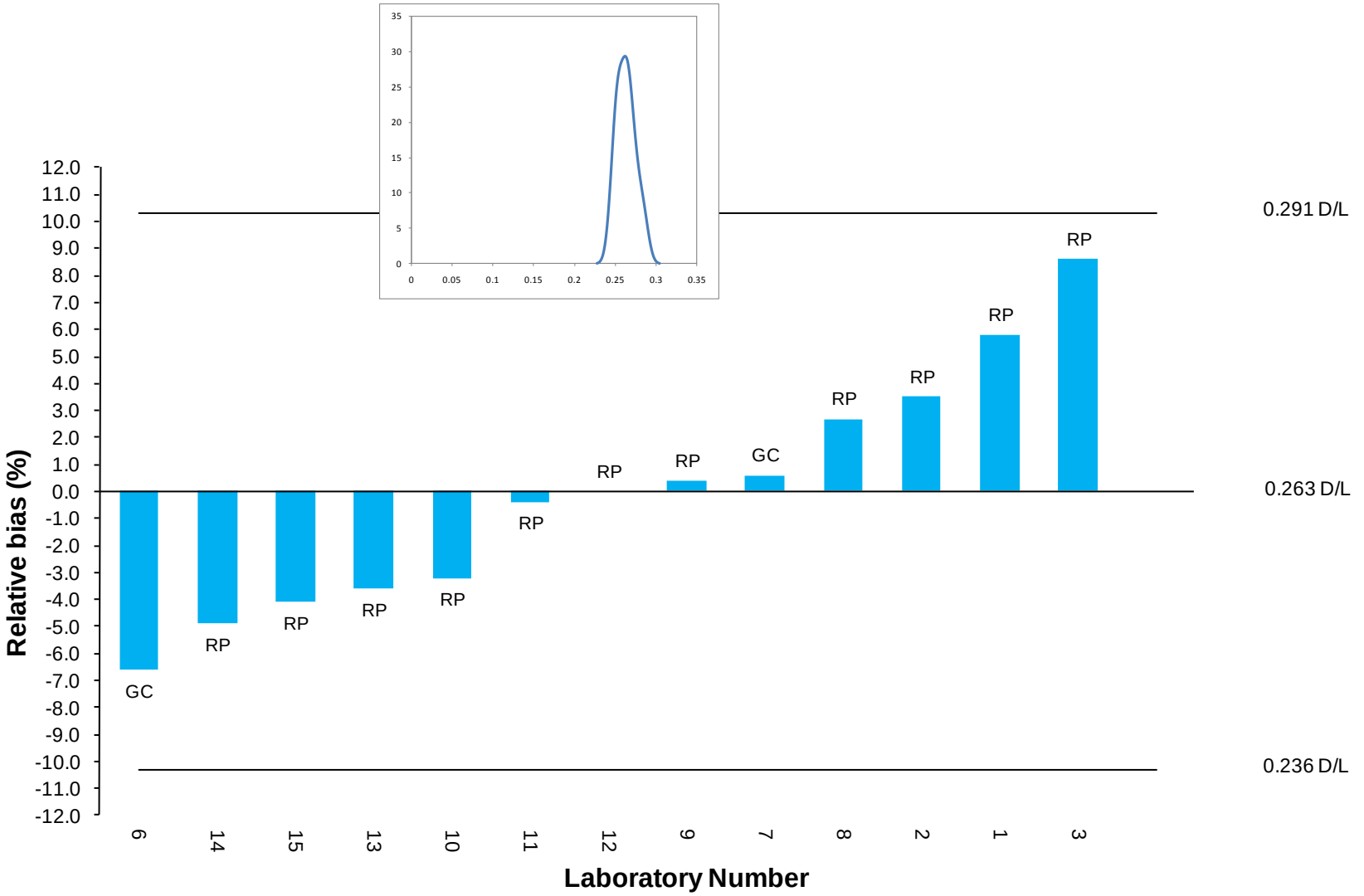


Figure 5.9: Relative Percentage Bias for **Alanine D/L Results (rpHPLC data only)** in Opercula Test Material

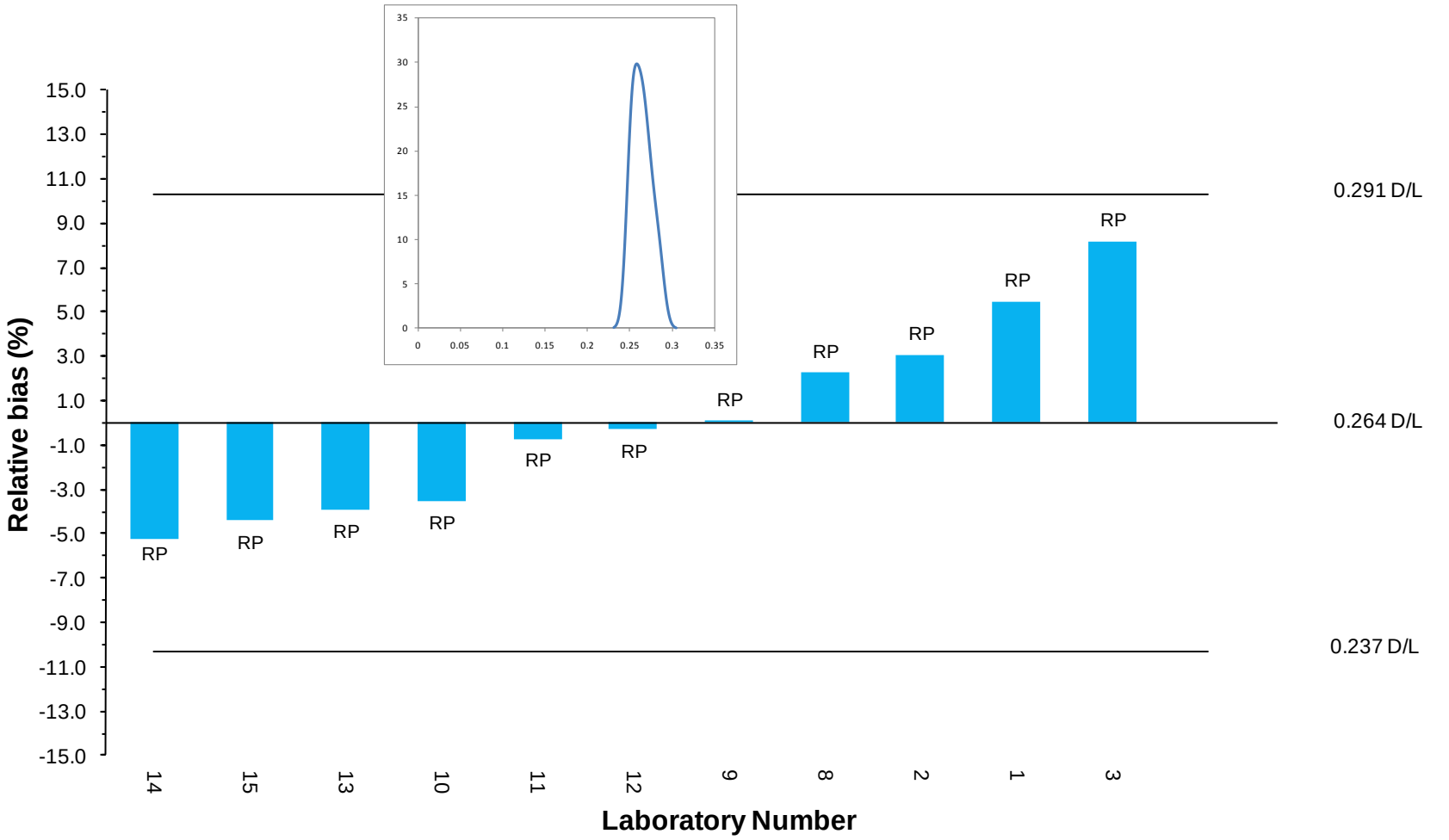


Figure 5.10: Relative Percentage Bias for **Valine D/L Results (all data)** in Opercula Test Material

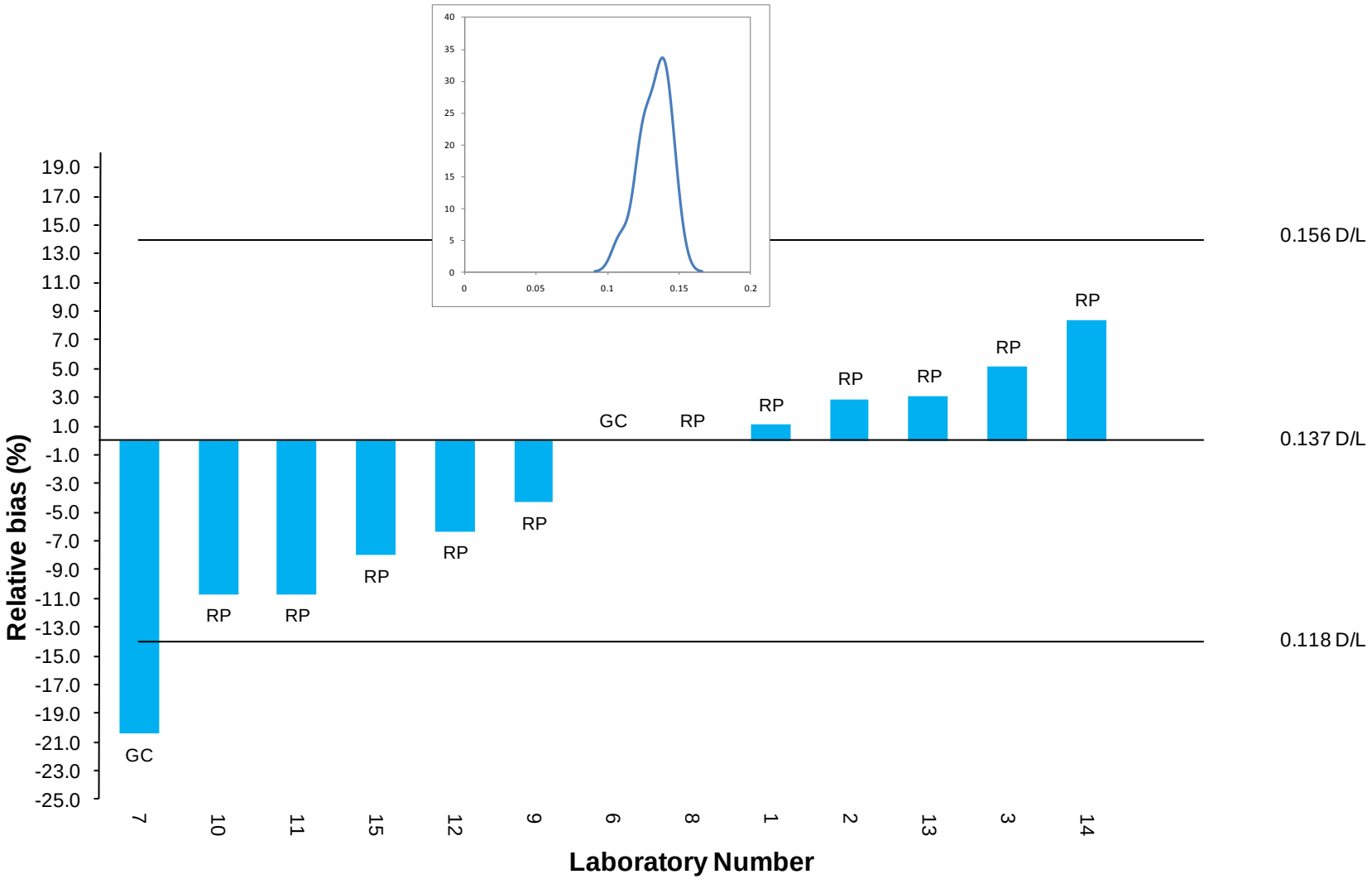


Figure 5.11: Relative Percentage Bias for **Valine D/L Results (rpHPLC data only)** in Opercula Test Material

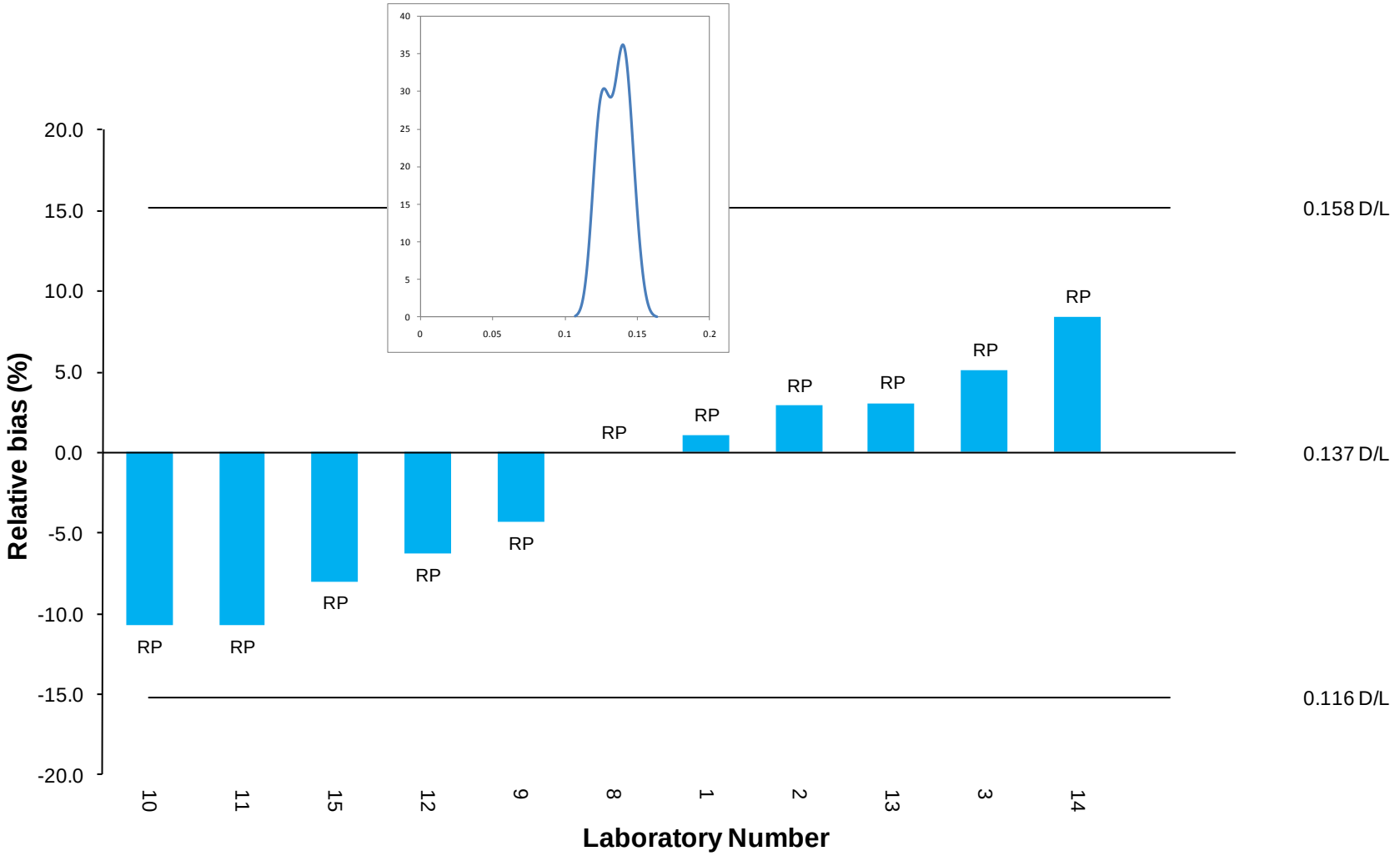


Figure 5.12: Relative Percentage Bias for **Phenylalanine D/L Results (all data)** in Opercula Test Material

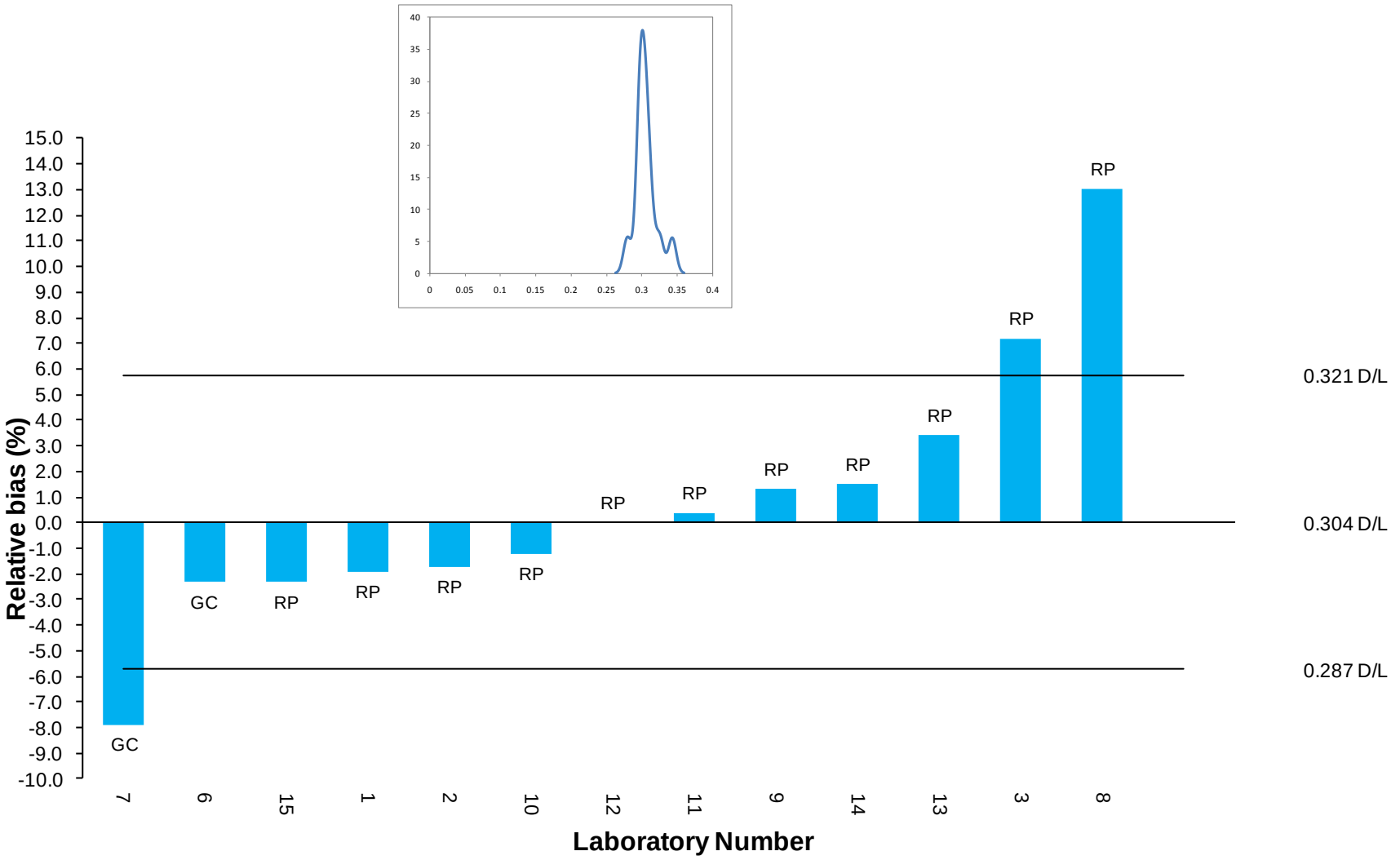


Figure 5.13: Relative Percentage Bias for **Phenylalanine D/L Results (rpHPLC data only)** in Opercula Test Material

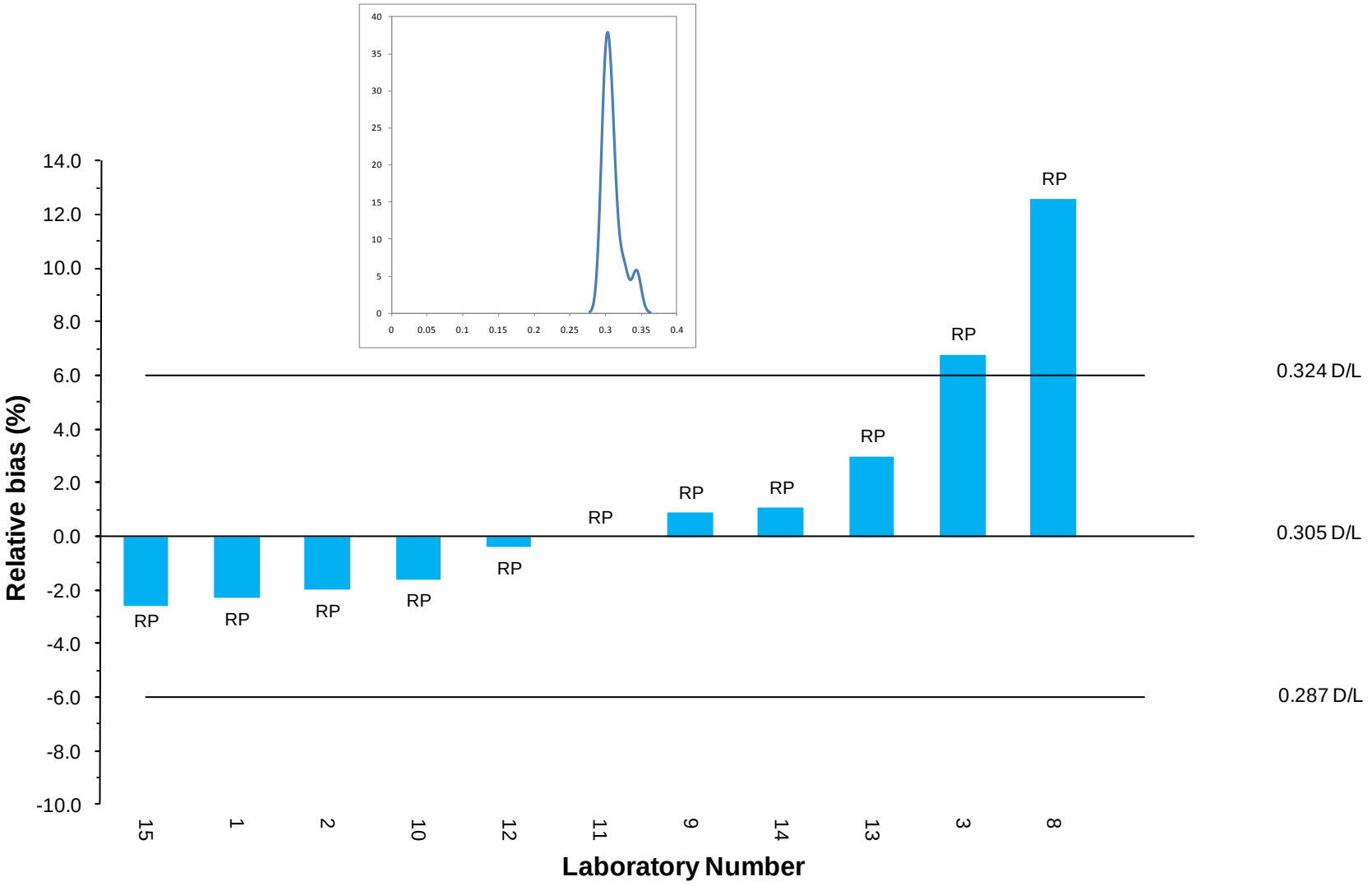


Figure 5.14: Relative Percentage Bias for **D-Alloisoleucine/L-Isoleucine Results (all data)** in Opercula Test Material

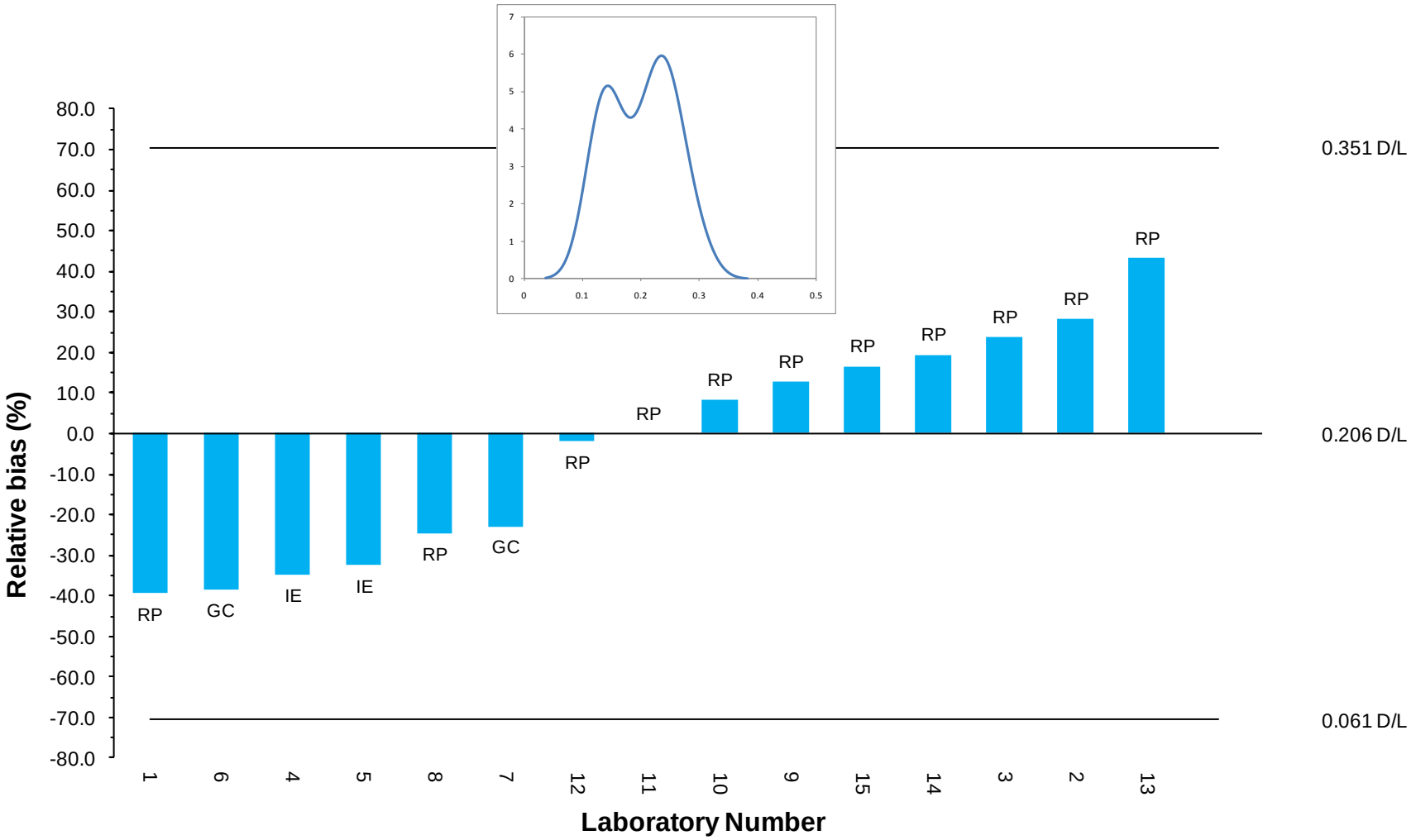


Figure 5.15: Relative Percentage Bias for **D-Alloisoleucine/L-Isoleucine Results (rpHPLC data only)** in Opercula Test Material

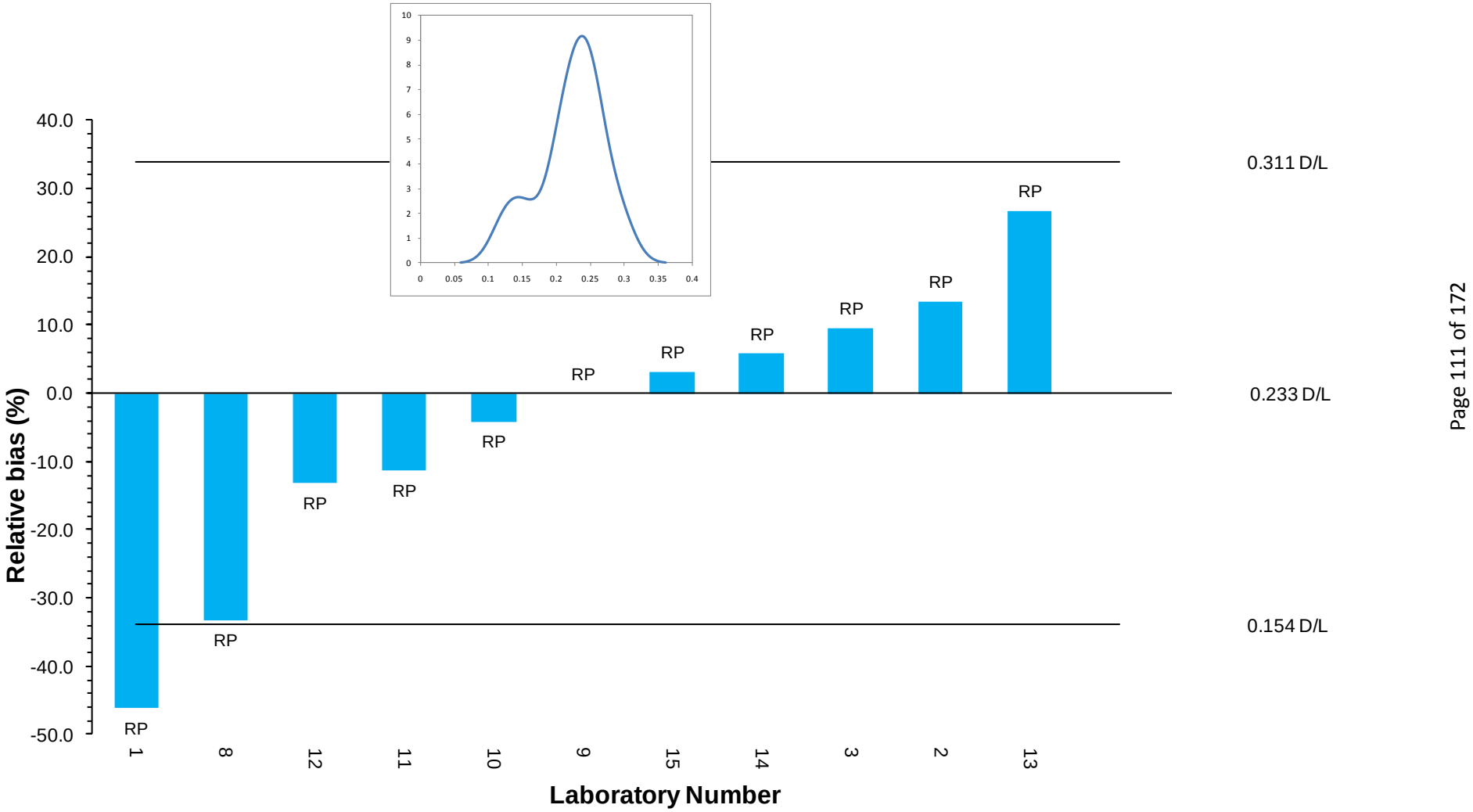


Figure 5.16: Relative Percentage Bias for **Leucine D/L Results (all data)** in Opercula Test Material

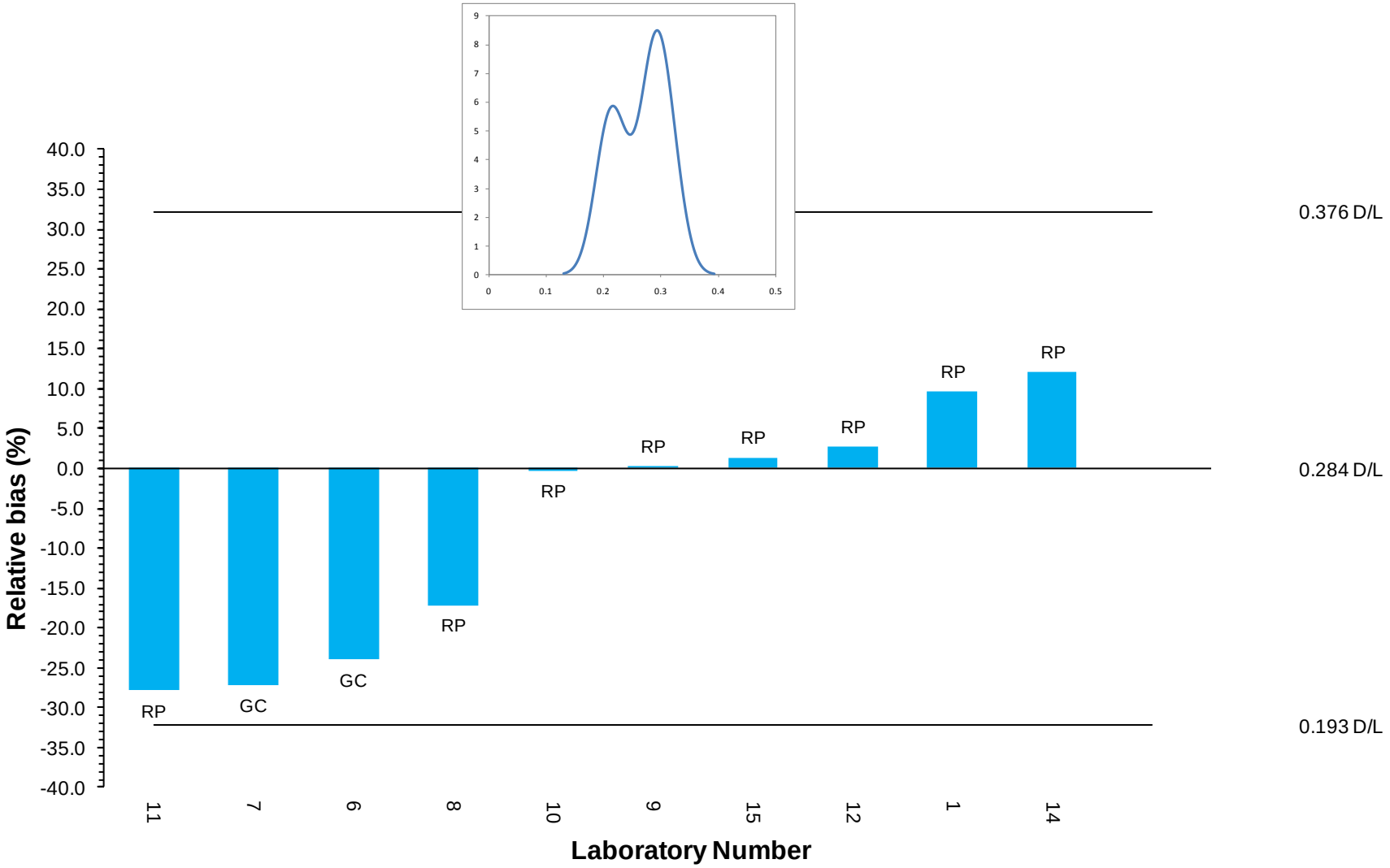


Figure 5.17: Relative Percentage Bias for **Leucine D/L Results (rpHPLC data only)** in Opercula Test Material

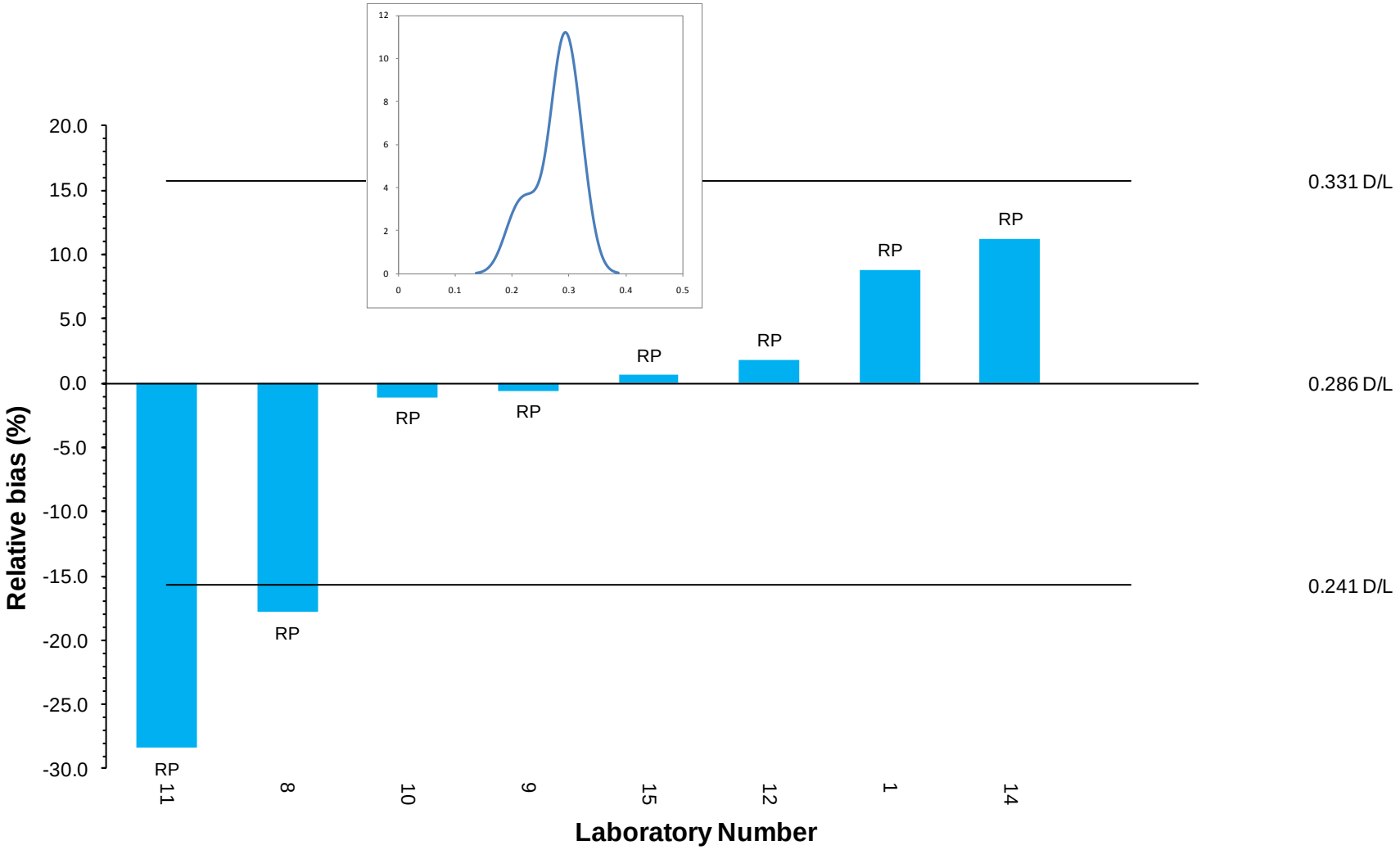
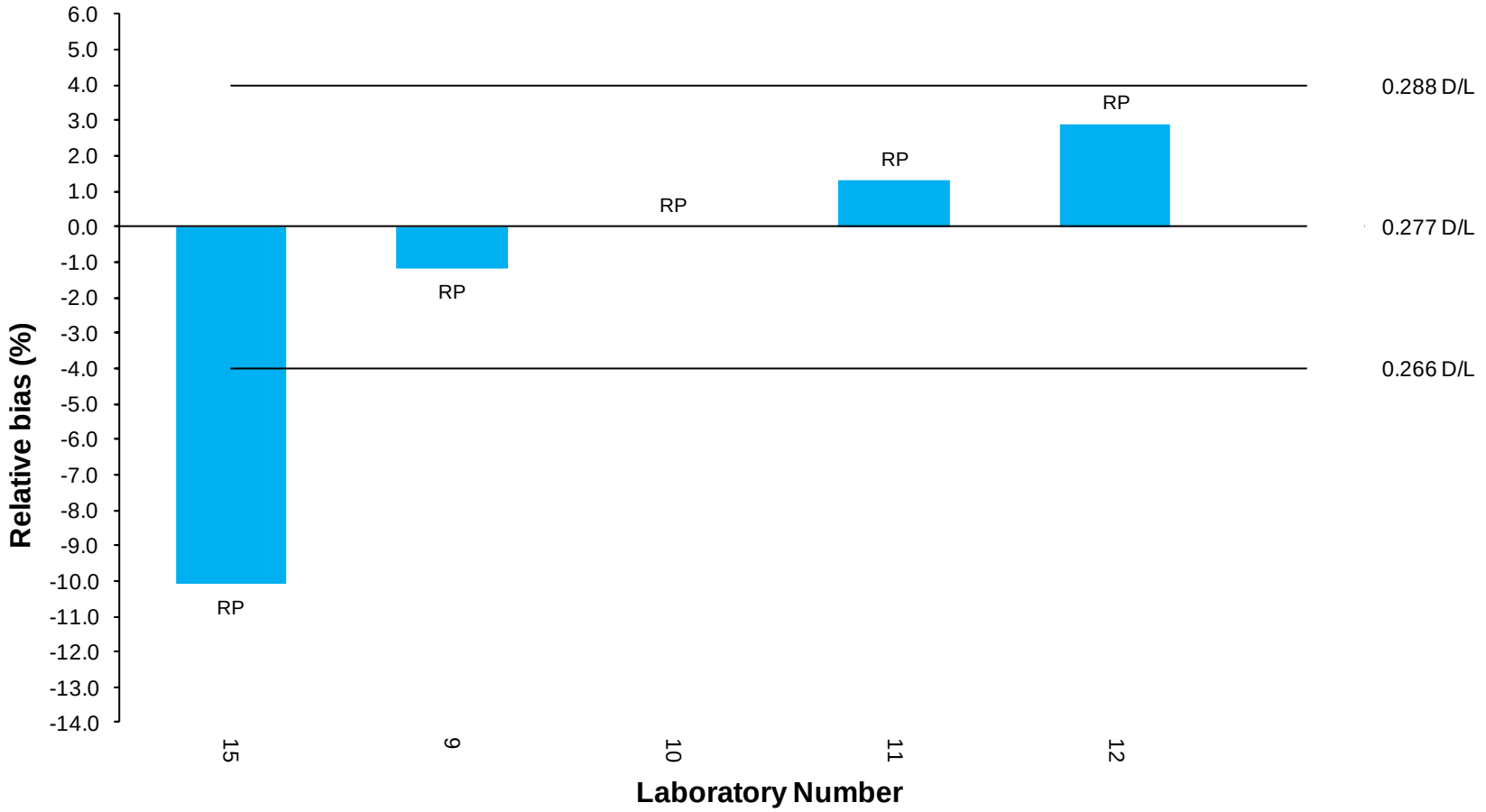


Figure 5.18: Relative Percentage Bias for **Tyrosine D/L Results (rpHPLC data only)** in Opercula Test Material



6 MEASUREMENT UNCERTAINTY
Opercula Test Material

6.1 Estimation of Measurement Uncertainty from Inter-laboratory comparisons.

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

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

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

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

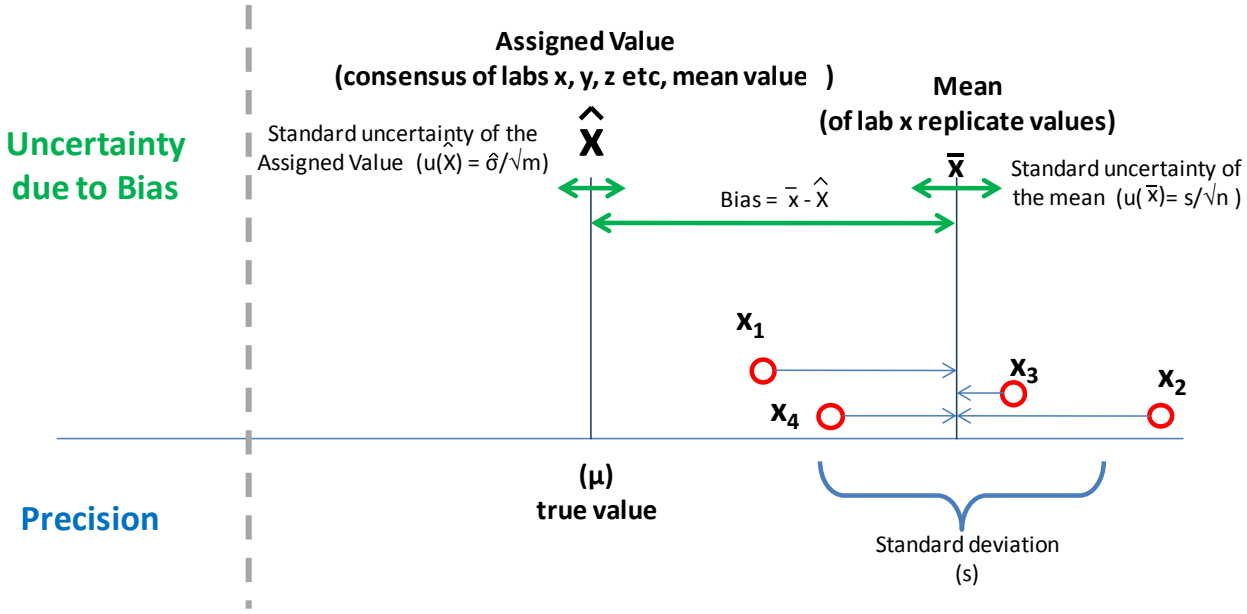
Thus; $$u_C = \sqrt{{S_{Rw}}^2 + u(bias)^2} \cong S_R$$

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

[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 ;

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

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

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

t is calculated as;

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

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

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

Thus, the bias uncertainty now becomes;

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

6.2.2 *For results from multiple proficiency tests*

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

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

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

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

6.3 Combined uncertainty (u_C).

The combined uncertainty is therefore calculated as;

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

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

Note concerning z-scores; for laboratories performing within the satisfactory range, i.e.; |z|=2, where there is a normal distribution of z-scores , that is, some may be positive and others negative, there will be no overall bias associated with the laboratory's performance. In this case the uncertainty associated with a

result will be based on the uncertainty of that result, i.e.; $u(\bar{x})$, plus the uncertainty of the assigned value $u(\hat{X})$, plus the precision contributionS_{Rw}, which in this case is equivalent to the target standard deviation, σ_p. Where the uncertainty of the assigned value and /or the uncertainty of the result is considered negligible compared to the target standard deviation used for assessment (σ_p), then the uncertainty associated with the laboratory's result is simply equivalent to σ_p, or it's RSD value expressed as a percentage.

6.4 Expanded Uncertainty (U).

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

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

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

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

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

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

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

6.5 Calculating Measurement Uncertainty for Amino Acids in Opercula Test Material

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

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

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

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

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

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

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

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

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

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

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

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

analyte	Std uncertainty contributions			Combined & Expanded uncertainties	
	Precision[1]	Bias components[2,3]			
	[1] $\hat{\sigma}$ as RSD%	[2] $u(\hat{X})$as RSU%	[3] RMS_{bias}%	combined u_c%	Expanded $U\%\ (k=2)$
Asx D/L (all[a])	1.02	0.28	4.97	5.08	10.16
Asx D/L (rpHPLC)	1.17	0.35	1.70	2.09	4.19
Glx D/L (all[a])	1.47	0.41	8.82	8.95	17.90
Glx D/L (rpHPLC)	1.29	0.39	6.41	6.55	13.10
Ser D/L (rpHPLC)	1.41	0.43	1.39	2.03	4.05
Arg D/L (rpHPLC)	21.76	7.25	22.2	31.92	63.83
Ala D/L (all[a])	5.16	1.43	4.25	6.83	13.66
Ala D/L (rpHPLC)	5.14	1.55	3.58	6.45	12.90
Val D/L (all[a])	6.99	1.94	8.25	10.99	21.98
Val D/L (rpHPLC)	7.58	2.28	5.5	9.64	19.27
Phe D/L (all[a])	2.87	0.79	4.94	5.77	11.53
Phe D/L (rpHPLC)	3.01	0.91	4.54	5.52	11.04
Aile/Ile D/L(all[b])	35.21	9.09	26.48	44.99	89.97
D-Aile/L-Ile (rpHPLC)	16.94	5.11	20.44	27.03	54.07
Leu D/L (all[a])	16.12	5.10	16.21	23.42	46.84
Leu D/L (rpHPLC)	7.86	2.78	12.9	15.36	30.72
Tyr D/L (rpHPLC)	1.99	0.89	4.78	5.25	10.50

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

[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]				
Asx D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
001	0.549	0.15	0.28	0.05	4.10	4.11	8.22
002	0.552	0.76	0.28	0.54	3.58	3.71	7.41
003	0.571	n=1	0.28	n=1	0.26		
004							
005							
006	0.650	n=1	0.28	n=1	13.54		
007	0.631	7.13	0.28	3.19	10.22	12.87	25.73
008	0.576	0.12	0.28	0.09	0.52	0.61	1.23
009	0.576	0.16	0.28	0.11	0.69	0.77	1.53
010	0.571	0.10	0.28	0.07	0.27	0.40	0.81
011	0.580	1.78	0.28	1.26	1.33	2.57	5.13
012	0.577	0.04	0.28	0.03	0.86	0.91	1.82
013	0.573	0.06	0.28	0.04	0.00	0.29	0.58
014	0.571	0.53	0.28	0.38	0.27	0.76	1.52
015	0.570	0.85	0.28	0.60	0.41	1.15	2.31
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 $U\%\ (k=2)$
001	0.549	0.15	0.35	0.05	3.95	3.97	7.95
002	0.552	0.76	0.35	0.54	3.44	3.58	7.15
003	0.571	n=1	0.35	n=1	0.12		
004							
005							
006	GC						
007	GC						
008	0.576	0.12	0.35	0.09	0.67	0.77	1.55
009	0.576	0.16	0.35	0.11	0.83	0.93	1.85
010	0.571	0.10	0.35	0.07	0.12	0.39	0.78
011	0.580	1.78	0.35	1.26	1.48	2.65	5.31
012	0.577	0.04	0.35	0.03	1.01	1.07	2.14
013	0.573	0.06	0.35	0.04	0.15	0.39	0.78
014	0.571	0.53	0.35	0.38	0.12	0.75	1.51
015	0.570	0.85	0.35	0.60	0.27	1.13	2.26

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

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]				
Glx D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
001	0.150	0.55	0.41	0.17	9.31	9.34	18.68
002	0.140	1.49	0.41	1.06	14.96	15.08	30.15
003	0.144	n=1	0.41	n=1	12.68		
004							
005							
006	0.202	n=1	0.41	n=1	22.48		
007	0.174	14.94	0.41	6.68	5.51	17.27	34.55
008	0.163	0.00	0.41	0.00	1.16	1.23	2.47
009	0.166	0.59	0.41	0.41	0.45	0.94	1.88
010	0.165	0.02	0.41	0.01	0.00	0.41	0.82
011	0.166	0.06	0.41	0.04	0.47	0.63	1.26
012	0.167	0.11	0.41	0.08	0.99	1.08	2.17
013	0.166	0.01	0.41	0.00	0.65	0.77	1.53
014	0.164	0.39	0.41	0.28	0.39	0.74	1.49
015	0.164	0.06	0.41	0.04	0.80	0.90	1.80
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 $U\%\ (k=2)$
001	0.150	0.55	0.39	0.17	8.96	8.99	17.97
002	0.140	1.49	0.39	1.06	14.63	14.75	29.49
003	0.144	n=1	0.39	n=1	12.34		
004							
005							
006	GC						
007	GC						
008	0.163	0.00	0.39	0.00	0.78	0.87	1.74
009	0.166	0.59	0.39	0.41	0.84	1.17	2.34
010	0.165	0.02	0.39	0.01	0.39	0.55	1.10
011	0.166	0.06	0.39	0.04	0.87	0.95	1.91
012	0.167	0.11	0.39	0.08	1.39	1.45	2.90
013	0.166	0.01	0.39	0.00	1.04	1.11	2.22
014	0.164	0.39	0.39	0.28	0.00	0.62	1.24
015	0.164	0.06	0.39	0.04	0.41	0.57	1.14

[4] = σ is the standard deviation of submitted results, expressed as a relative % i.e.; $CV\% = (\sigma/\bar{x}) \times 100$ (see Section 4).
[5] =u$(\hat{X})$ is the uncertainty of the assigned value $(\hat{X})$ expressed as a relative % i.e.; $RSU_{\hat{X}}\% = (\mathrm{u}(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = u$(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\hat{x}}\% = (\mathrm{u}(\bar{x})/\bar{x}) \times 100$ (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]				
Ser D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
001	0.647	0.99	0.43	0.31	2.21	2.48	4.96
002	0.662	0.35	0.43	0.25	0.00	0.61	1.21
003	0.667	n=1	0.43	n=1	0.81		
004							
005							
006							
007							
008	0.673	0.21	0.43	0.15	1.71	1.79	3.57
009	0.663	0.70	0.43	0.49	0.21	0.98	1.95
010	0.644	2.55	0.43	1.80	2.74	4.18	8.36
011	0.653	1.65	0.43	1.17	1.27	2.43	4.86
012	0.667	0.53	0.43	0.37	0.76	1.09	2.17
013	0.668	0.16	0.43	0.11	0.94	1.05	2.10
014	0.655	1.83	0.43	1.30	0.95	2.48	4.95
015	0.655	0.78	0.43	0.55	1.05	1.48	2.96
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 $U\%\ (k=2)$
001							
002	0.979	1.40	7.25	0.99	21.88	23.11	46.22
003	0.921	n=1	7.25	n=1	14.67		
004							
005							
006							
007							
008							
009	0.860	31.71	7.25	22.42	7.08	40.14	80.28
010	0.796	34.72	7.25	24.55	0.86	43.15	86.29
011	0.668	13.54	7.25	9.58	16.85	24.73	49.46
012	0.713	18.93	7.25	13.39	11.18	26.75	53.49
013	0.366	13.70	7.25	9.69	54.38	57.37	114.75
014	0.948	4.43	7.25	3.13	18.04	20.19	40.37
015	0.803	5.42	7.25	3.83	0.00	9.83	19.66

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

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]				
Ala D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
001	0.279	1.58	1.43	0.50	5.83	6.23	12.46
002	0.273	1.58	1.43	1.12	3.48	4.23	8.45
003	0.286	n=1	1.43	n=1	8.56		
004							
005							
006	0.246	n=1	1.43	n=1	6.63		
007	0.265	3.40	1.43	1.28	0.59	3.95	7.89
008	0.271	0.26	1.43	0.18	2.67	3.05	6.10
009	0.265	0.95	1.43	0.67	0.42	1.89	3.78
010	0.255	1.92	1.43	1.36	3.20	4.22	8.44
011	0.262	2.36	1.43	1.67	0.41	3.25	6.51
012	0.263	1.80	1.43	1.27	0.00	2.62	5.25
013	0.254	0.08	1.43	0.06	3.59	3.87	7.73
014	0.251	4.42	1.43	3.13	4.87	7.43	14.85
015	0.253	4.18	1.43	2.96	4.06	6.69	13.37
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 $U\%\ (k=2)$
001	0.279	1.58	1.55	0.50	5.49	5.94	11.88
002	0.273	1.58	1.55	1.12	3.14	4.00	8.00
003	0.286	n=1	1.55	n=1	8.20		
004							
005							
006							
007							
008	0.271	0.26	1.55	0.18	2.34	2.82	5.64
009	0.265	0.95	1.55	0.67	0.09	1.94	3.87
010	0.255	1.92	1.55	1.36	3.52	4.50	9.01
011	0.262	2.36	1.55	1.67	0.73	3.36	6.73
012	0.263	1.80	1.55	1.27	0.33	2.71	5.42
013	0.254	0.08	1.55	0.06	3.91	4.20	8.41
014	0.251	4.42	1.55	3.13	5.18	7.66	15.32
015	0.253	4.18	1.55	2.96	4.37	6.91	13.82

[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.; $RSU_{\hat{X}}\% = (\mathrm{u}(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = u$(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\hat{x}}\% = (\mathrm{u}(\bar{x})/\bar{x}) \times 100$ (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]				
Val D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
001	0.139	2.64	1.94	0.84	1.14	3.57	7.14
002	0.141	0.65	1.94	0.46	2.90	3.58	7.16
003	0.144	n=1	1.94	n=1	5.11		
004							
005							
006	0.137	n=1	1.94	n=1	0.00		
007	0.109	5.50	1.94	1.83	20.44	21.33	42.67
008	0.137	0.00	1.94	0.00	0.00	1.94	3.88
009	0.131	1.31	1.94	0.92	4.33	5.00	10.01
010	0.122	1.79	1.94	1.27	10.74	11.13	22.26
011	0.122	1.02	1.94	0.72	10.69	10.94	21.88
012	0.128	6.41	1.94	4.53	6.27	10.23	20.47
013	0.141	0.26	1.94	0.18	3.08	3.66	7.31
014	0.149	13.27	1.94	9.38	8.40	18.39	36.79
015	0.126	4.37	1.94	3.09	7.98	9.80	19.60
Val D/L rpHPLC		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
001	0.139	2.64	2.28	0.84	1.14	3.77	7.54
002	0.141	0.65	2.28	0.46	2.90	3.78	7.55
003	0.144	n=1	2.28	n=1	5.11		
004							
005							
006							
007							
008	0.137	0.00	2.28	0.00	0.00	2.28	4.57
009	0.131	1.31	2.28	0.92	4.33	5.15	10.29
010	0.122	1.79	2.28	1.27	10.74	11.20	22.39
011	0.122	1.02	2.28	0.72	10.69	11.01	22.01
012	0.128	6.41	2.28	4.53	6.27	10.30	20.61
013	0.141	0.26	2.28	0.18	3.08	3.85	7.70
014	0.149	13.27	2.28	9.38	8.40	18.43	36.86
015	0.126	4.37	2.28	3.09	7.98	9.87	19.75

[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.; $RSU_{\hat{X}}\% = (\mathrm{u}(\hat{X})/\hat{X}) \times 100$ (see Section 5)

[6] = u$(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\hat{x}}\% = (\mathrm{u}(\bar{x})/\bar{x}) \times 100$ (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]				
Phe D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
001	0.298	6.61	0.79	2.09	1.93	7.24	14.48
002	0.299	0.41	0.79	0.29	1.66	1.91	3.82
003	0.326	n=1	0.79	n=1	7.22		
004							
005							
006	0.297	n=1	0.79	n=1	2.32		
007	0.280	10.71	0.79	3.39	7.91	13.77	27.53
008	0.344	0.62	0.79	0.44	12.97	13.02	26.04
009	0.308	0.52	0.79	0.37	1.32	1.67	3.33
010	0.300	0.75	0.79	0.53	1.25	1.74	3.48
011	0.305	0.56	0.79	0.40	0.37	1.12	2.24
012	0.304	1.41	0.79	0.99	0.00	1.90	3.79
013	0.314	0.57	0.79	0.41	3.40	3.56	7.11
014	0.309	10.14	0.79	7.17	1.48	12.53	25.06
015	0.297	0.12	0.79	0.08	2.28	2.42	4.85
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 $U\%\ (k=2)$
001	0.298	6.61	0.91	2.09	2.30	7.36	14.72
002	0.299	0.41	0.91	0.29	2.03	2.28	4.55
003	0.326	n=1	0.91	n=1	6.82		
004							
005							
006							
007							
008	0.344	0.62	0.91	0.44	12.56	12.61	25.22
009	0.308	0.52	0.91	0.37	0.94	1.46	2.91
010	0.300	0.75	0.91	0.53	1.62	2.07	4.13
011	0.305	0.56	0.91	0.40	0.00	1.14	2.28
012	0.304	1.41	0.91	0.99	0.37	1.98	3.96
013	0.314	0.57	0.91	0.41	3.01	3.22	6.45
014	0.309	10.14	0.91	7.17	1.10	12.50	25.00
015	0.297	0.12	0.91	0.08	2.65	2.80	5.60

[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.; $RSU_{\hat{X}}\% = (\mathrm{u}(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = u$(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\hat{x}}\% = (\mathrm{u}(\bar{x})/\bar{x}) \times 100$ (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]				
D-Aile/L-Ile		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
001	0.125	7.47	9.09	2.36	39.27	41.06	82.13
002	0.264	2.58	9.09	1.82	28.17	29.76	59.53
003	0.255	n=1	9.09	n=1	23.75		
004	0.135	1.58	9.09	1.12	34.73	35.95	71.90
005	0.139	3.05	9.09	2.16	32.54	34.00	67.99
006	0.127	n=1	9.09	n=1	38.37		
007	0.159	8.81	9.09	2.78	22.84	26.26	52.52
008	0.156	0.45	9.09	0.32	24.54	26.17	52.35
009	0.233	25.16	9.09	17.79	12.90	34.62	69.24
010	0.223	26.21	9.09	18.53	8.25	34.37	68.74
011	0.206	17.96	9.09	12.70	0.00	23.80	47.60
012	0.202	16.93	9.09	11.97	1.98	22.72	45.45
013	0.295	2.29	9.09	1.62	43.17	44.20	88.40
014	0.246	18.63	9.09	13.17	19.54	31.39	62.77
015	0.240	7.11	9.09	5.03	16.56	20.80	41.61
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 $U\%\ (k=2)$
001	0.125	7.47	5.11	2.36	46.21	47.14	94.29
002	0.264	2.58	5.11	1.82	13.52	14.80	29.59
003	0.255	n=1	5.11	n=1	9.61		
004	IE						
005	IE						
006	GC						
007	GC						
008	0.156	0.45	5.11	0.32	33.16	33.55	67.11
009	0.233	25.16	5.11	17.79	0.00	31.24	62.47
010	0.223	26.21	5.11	18.53	4.12	32.77	65.53
011	0.206	17.96	5.11	12.70	11.42	25.31	50.62
012	0.202	16.93	5.11	11.97	13.18	25.09	50.18
013	0.295	2.29	5.11	1.62	26.81	27.43	54.87
014	0.246	18.63	5.11	13.17	5.88	24.11	48.22
015	0.240	7.11	5.11	5.03	3.25	10.60	21.21

[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.; $RSU_{\hat{X}}\% = (\mathrm{u}(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = u$(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\hat{x}}\% = (\mathrm{u}(\bar{x})/\bar{x}) \times 100$ (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]				
Leu D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
001	0.311	8.93	5.10	2.83	9.65	14.38	28.77
002							
003							
004							
005							
006	0.216	n=1	5.10	n=1	23.96		
007	0.207	2.90	5.10	0.92	27.13	27.77	55.54
008	0.236	2.70	5.10	1.91	17.10	18.15	36.29
009	0.285	5.34	5.10	3.78	0.25	8.30	16.59
010	0.283	3.22	5.10	2.28	0.25	6.45	12.90
011	0.205	6.49	5.10	4.59	27.84	29.40	58.80
012	0.292	1.49	5.10	1.06	2.69	6.05	12.09
013							
014	0.318	10.53	5.10	7.45	12.09	18.40	36.79
015	0.288	3.88	5.10	2.74	1.40	7.11	14.21
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 $U\%\ (k=2)$
001	0.311	8.93	2.78	2.83	8.75	13.12	26.24
002							
003							
004							
005							
006	GC						
007	GC						
008	0.236	2.70	2.78	1.91	17.78	18.30	36.59
009	0.285	5.34	2.78	3.78	0.57	7.13	14.26
010	0.283	3.22	2.78	2.28	1.07	4.94	9.89
011	0.205	6.49	2.78	4.59	28.44	29.66	59.31
012	0.292	1.49	2.78	1.06	1.85	3.80	7.61
013							
014	0.318	10.53	2.78	7.45	11.17	17.28	34.57
015	0.288	3.88	2.78	2.74	0.57	5.53	11.07

[4] = σ is the standard deviation of submitted results, expressed as a relative % i.e.; $CV\% = (\sigma/\bar{x}) \times 100$ (see Section 4).
[5] =u$(\hat{X})$ is the uncertainty of the assigned value $(\hat{X})$ expressed as a relative % i.e.; $RSU_{\hat{X}}\% = (\mathrm{u}(\hat{X})/\hat{X}) \times 100$ (see Section 5)
[6] = u$(\bar{x})$ is the bias standard deviation for submitted results $(\bar{x})$ expressed as a relative % $RSU_{\hat{x}}\% = (\mathrm{u}(\bar{x})/\bar{x}) \times 100$ (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]				
Try D/L		σ std dev as CV%[4]	$u(\hat{X})$as RSU%[5]	$u(\bar{x})$as RSU%[6]	Relative bias %[7]	combined u_c%	Expanded $U\%\ (k=2)$
001							
002							
003							
004							
005							
006							
007							
008							
009	0.274	3.24	0.89	2.29	1.17	4.23	8.46
010	0.277	3.57	0.89	2.52	0.00	4.46	8.92
011	0.281	0.25	0.89	0.18	1.34	1.64	3.28
012	0.285	0.07	0.89	0.05	2.93	3.06	6.13
013							
014							
015	0.249	1.27	0.89	0.90	10.14	10.29	20.58

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

Figure 6.2: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Aspartic acid / Asparagine** D/L Values in Opercula Test Material

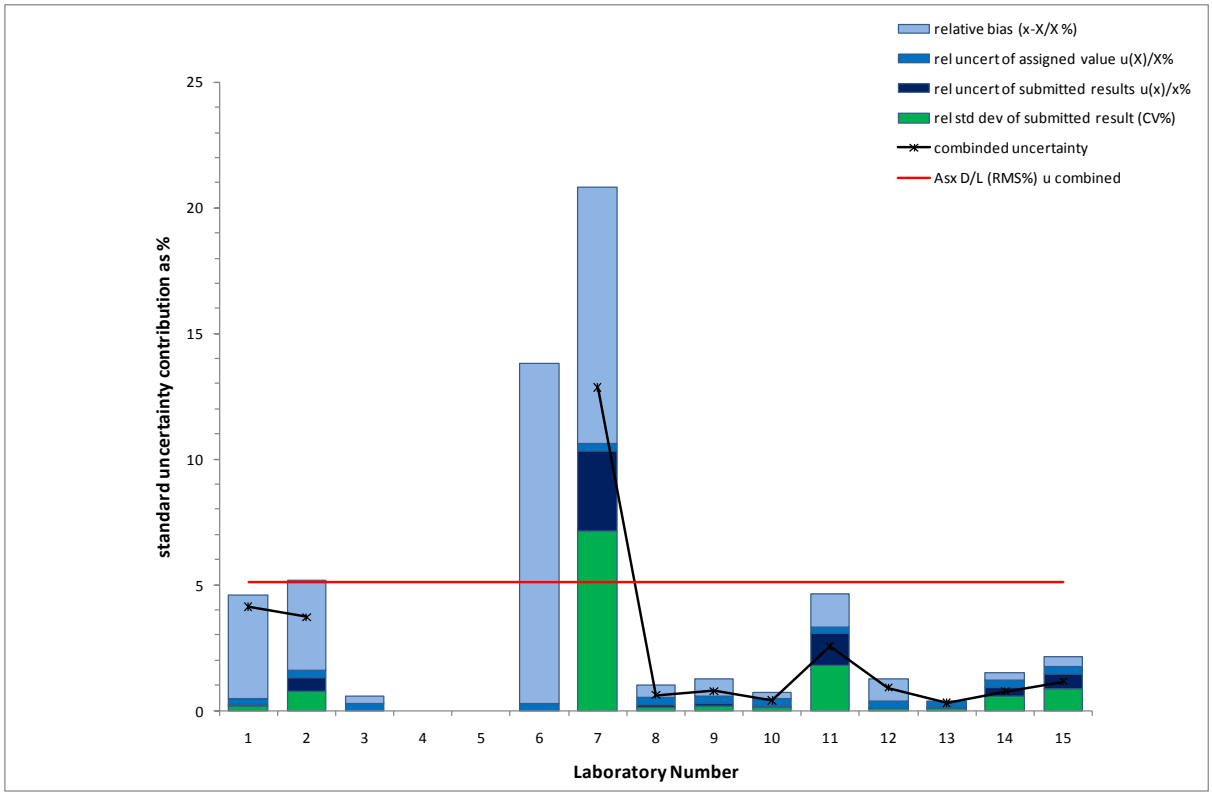


Figure 6.3: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Aspartic acid / Asparagine** D/L Values in Opercula Test Material

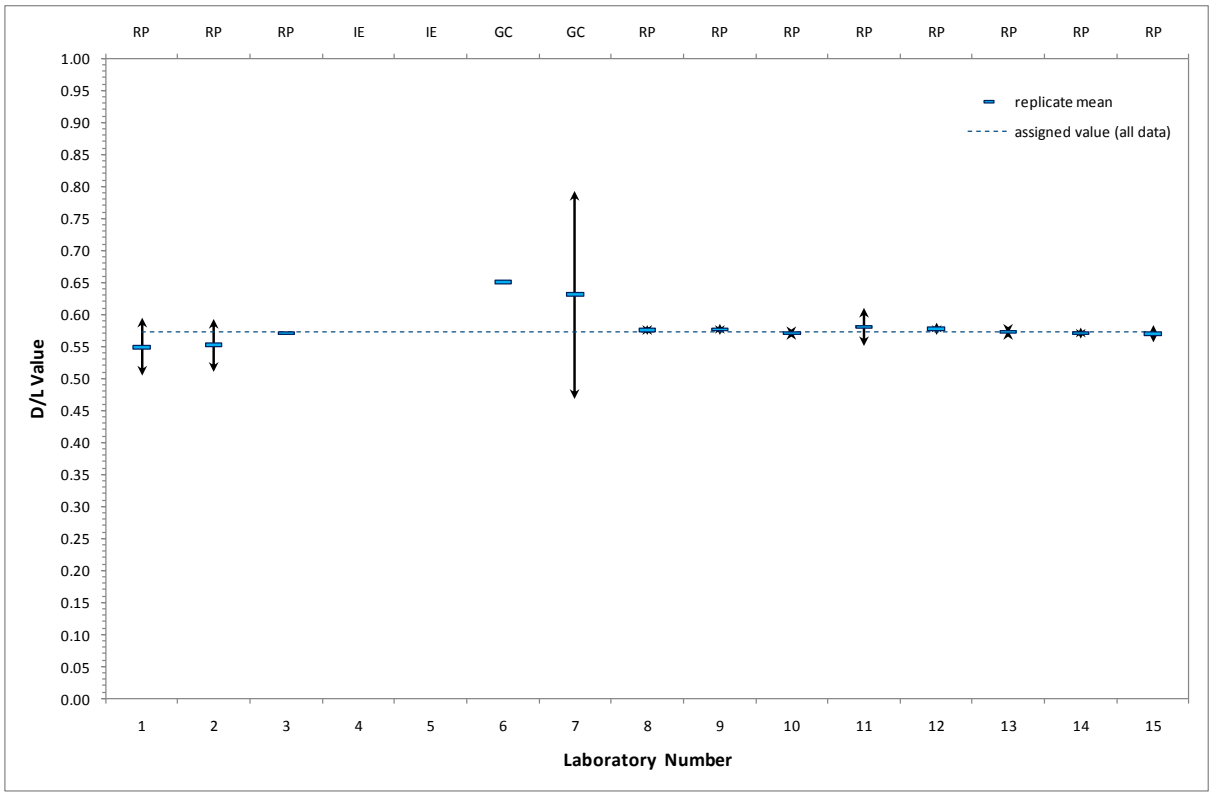


Figure 6.4: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Aspartic acid / Asparagine rpHPLC** D/L Values in Opercula Test Material

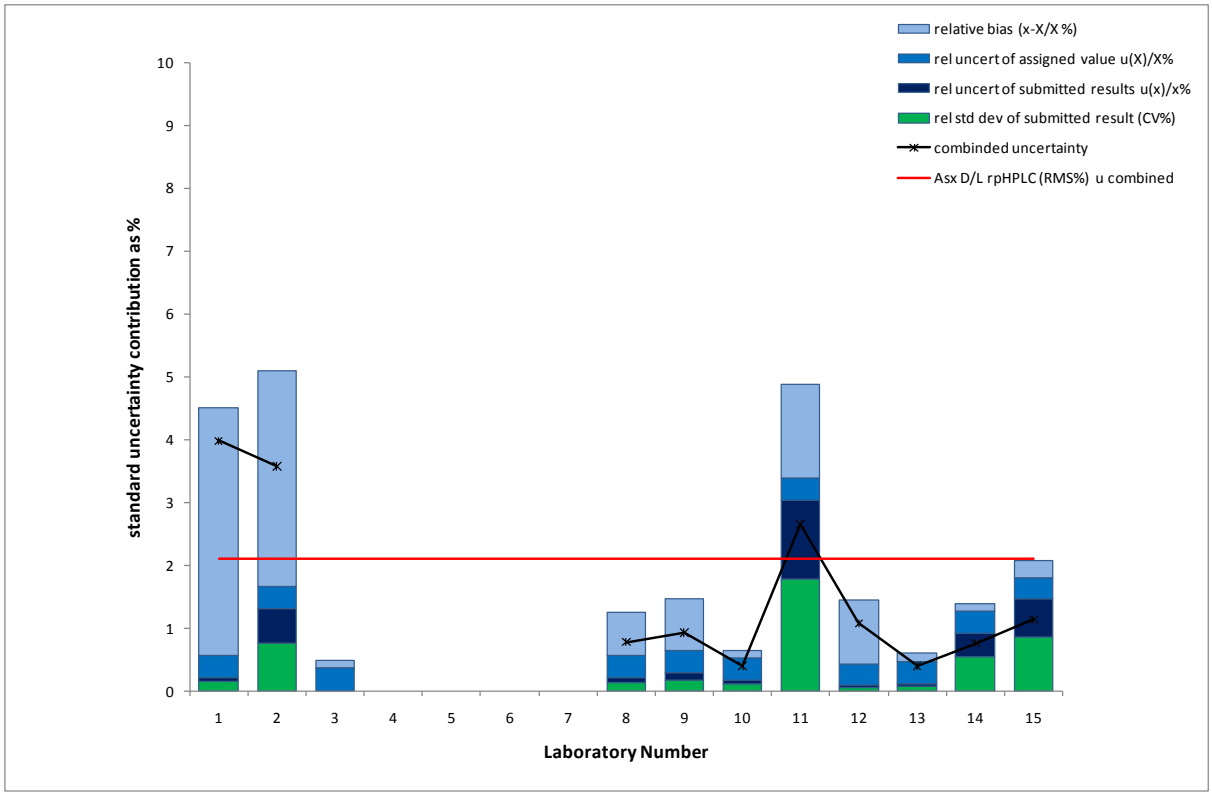


Figure 6.5: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Aspartic acid / Asparagine rpHPLC** D/L Values in Opercula Test Material

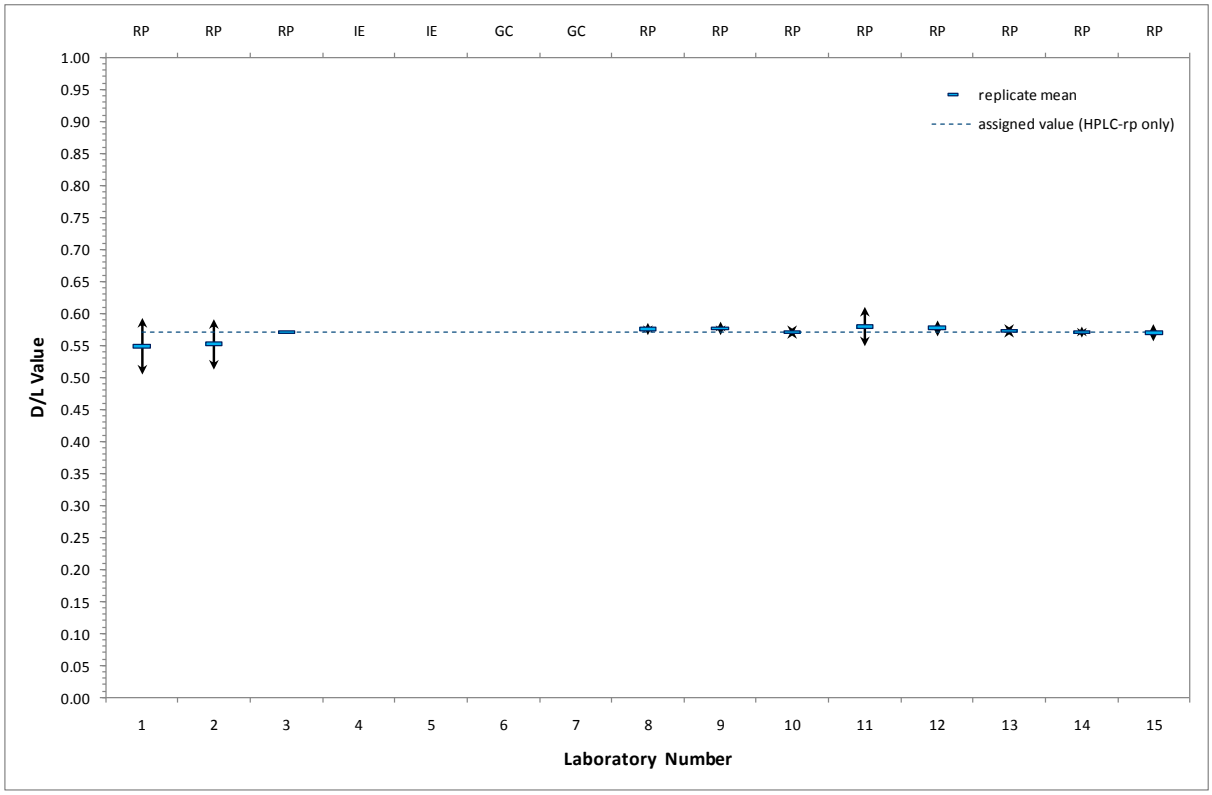


Figure 6.6: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Glutamic acid / Glutamine** D/L Values in Opercula Test Material

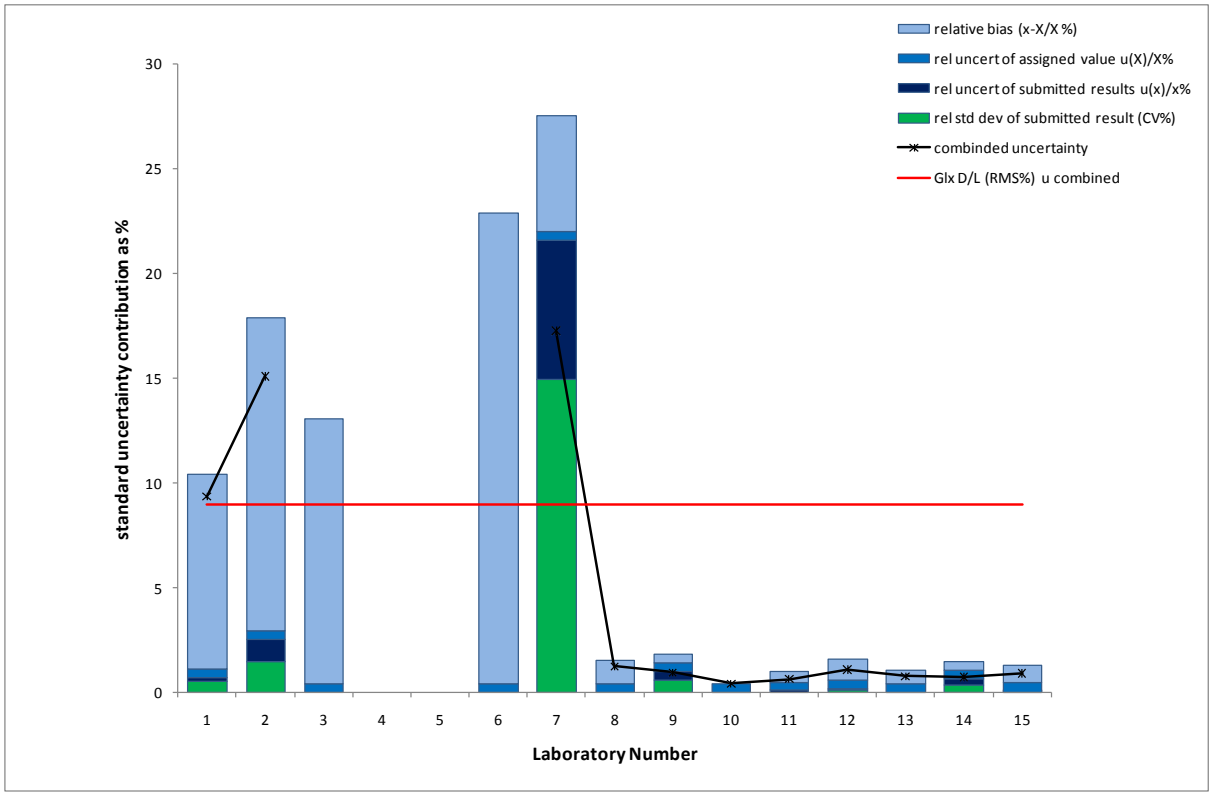


Figure 6.7: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Glutamic acid / Glutamine** D/L Values in Opercula Test Material

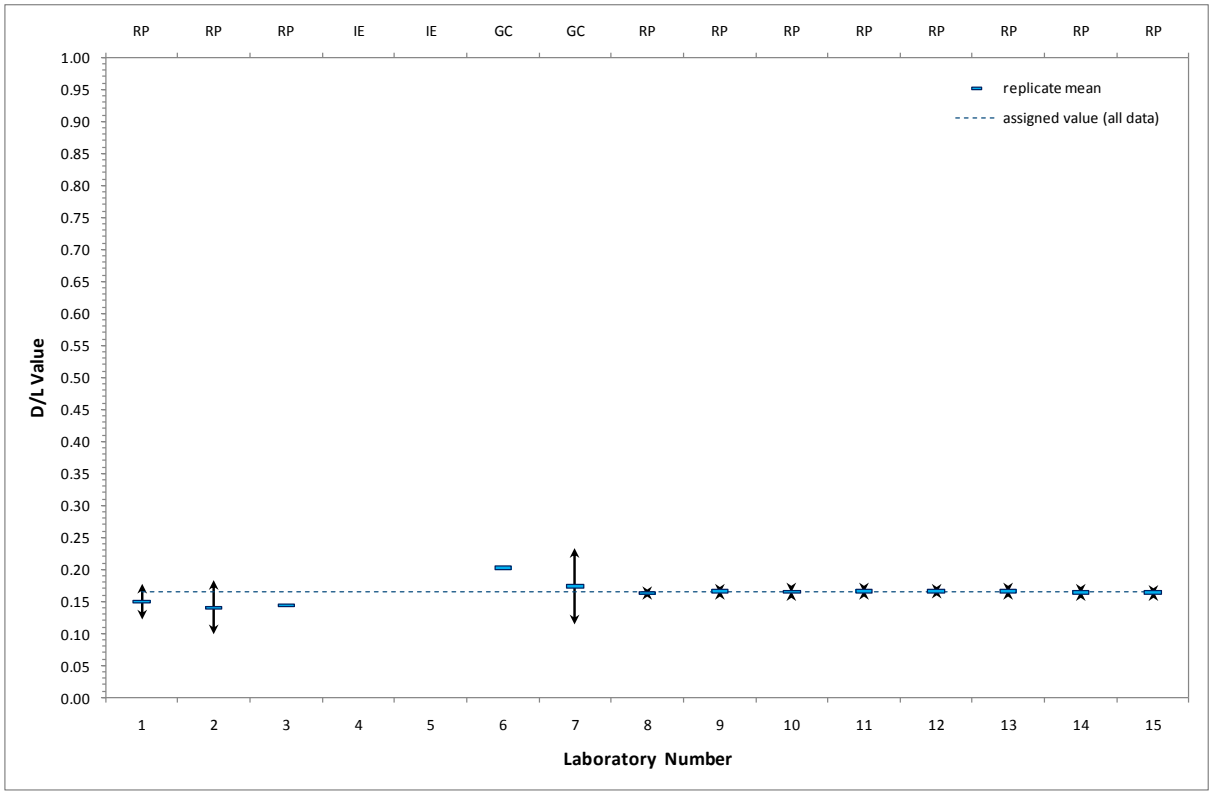


Figure 6.8: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Glutamic acid / Glutamine rpHPLC** D/L Values in Opercula Test Material

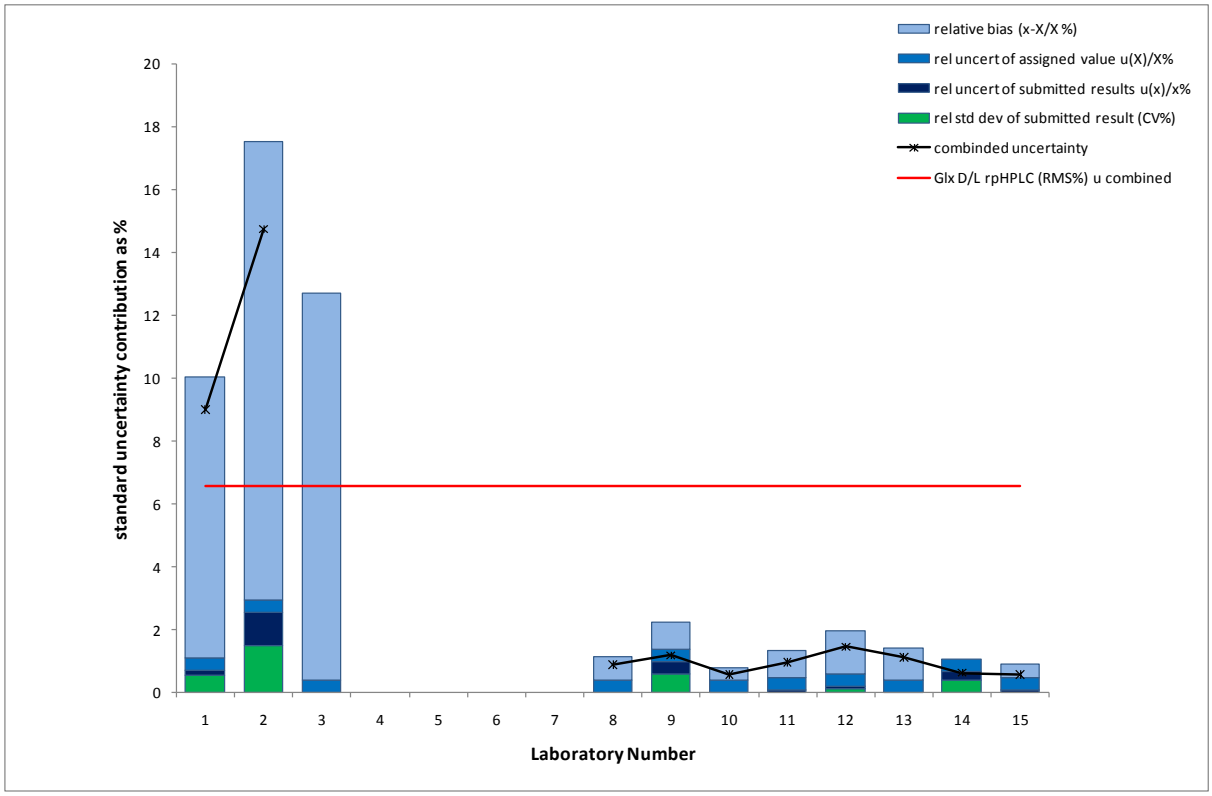


Figure 6.9: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Glutamic acid / Glutamine rpHPLC** D/L Values in Opercula Test Material

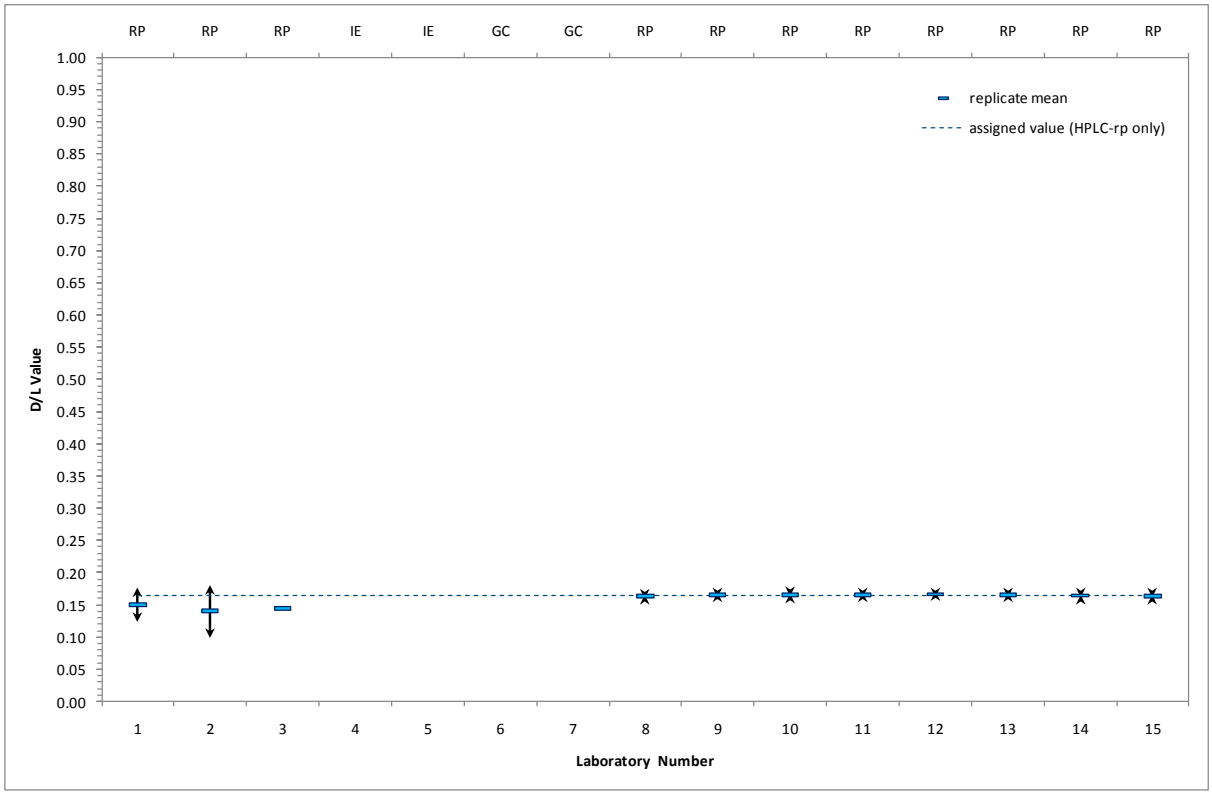


Figure 6.10: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Serine** D/L Values in Opercula Test Material

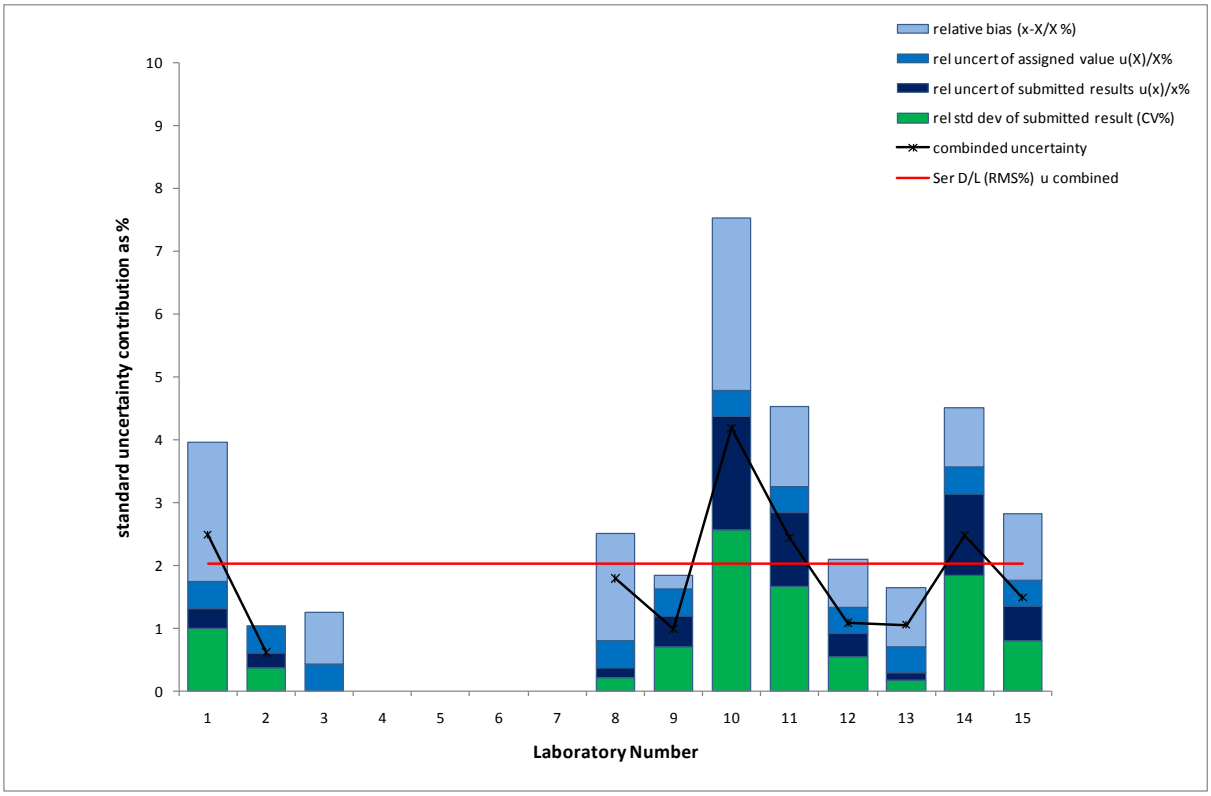


Figure 6.11: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Serine** D/L Values in Opercula Test Material

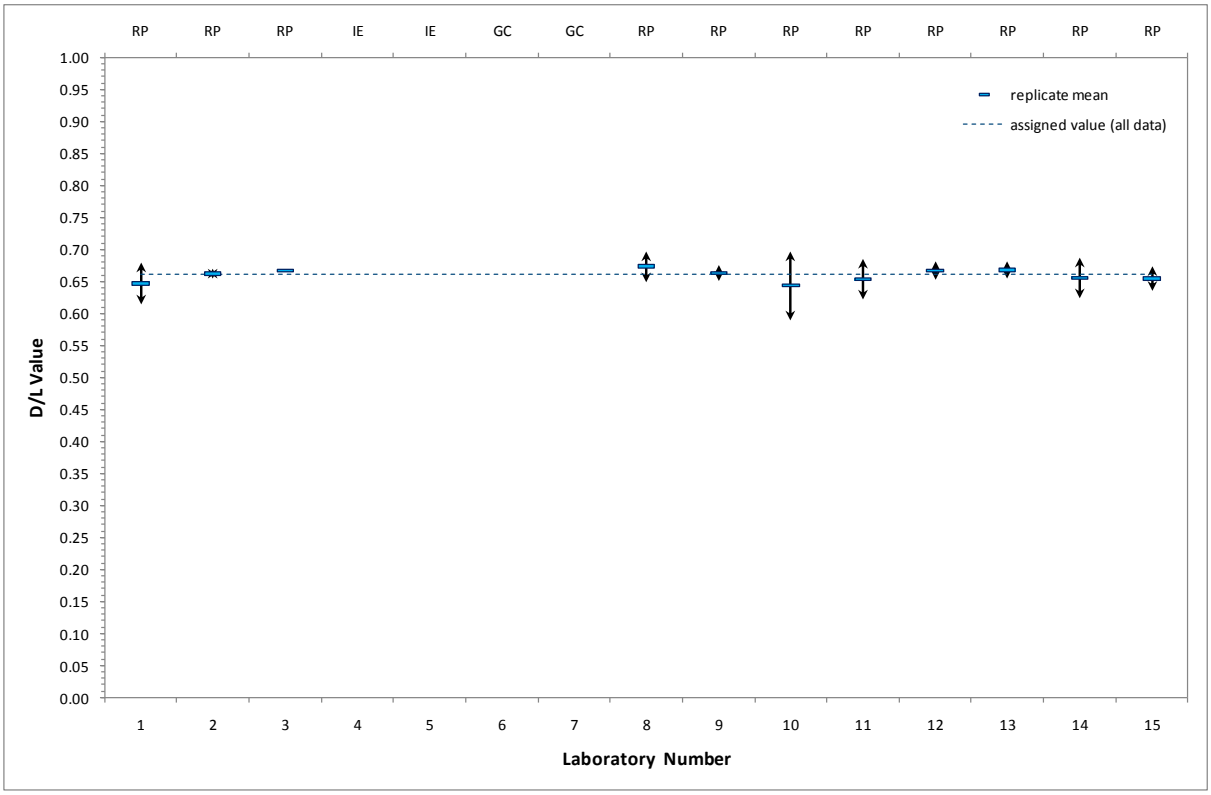


Figure 6.12: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Arginine** D/L Values in Opercula Test Material

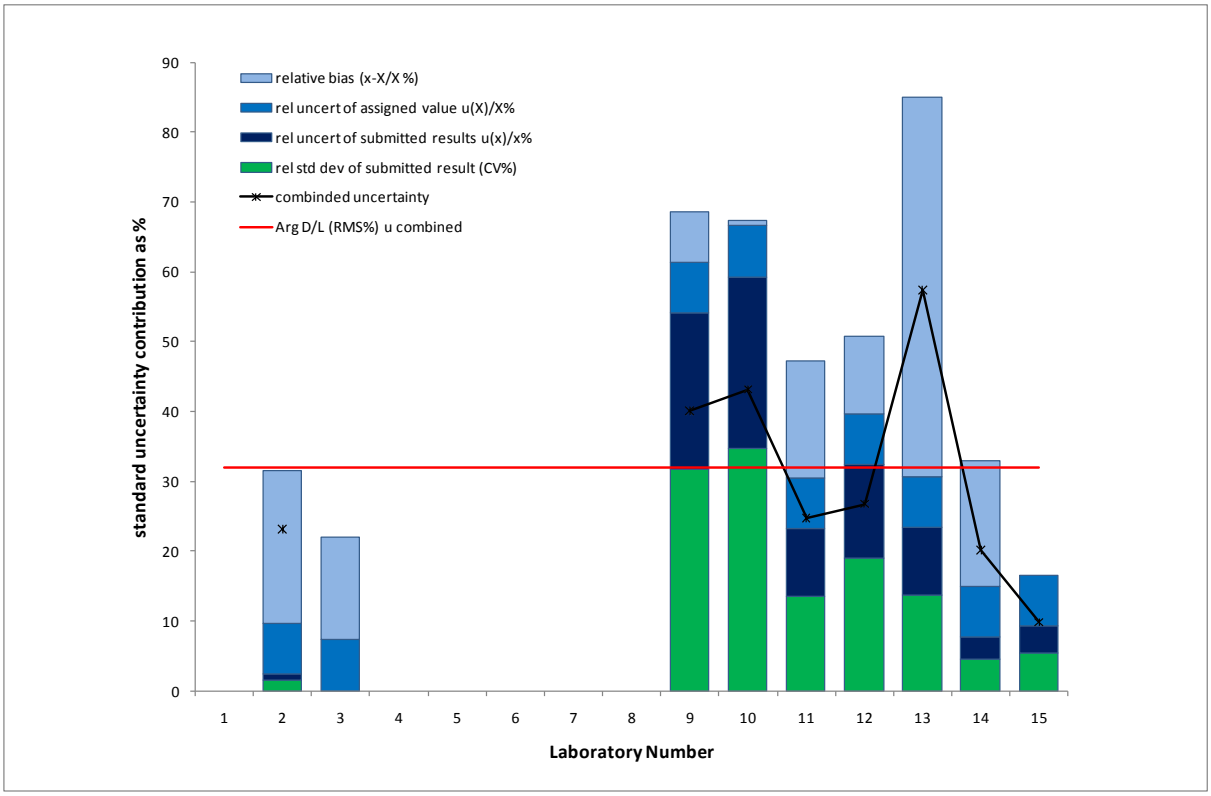


Figure 6.13: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Arginine** D/L Values in Opercula Test Material

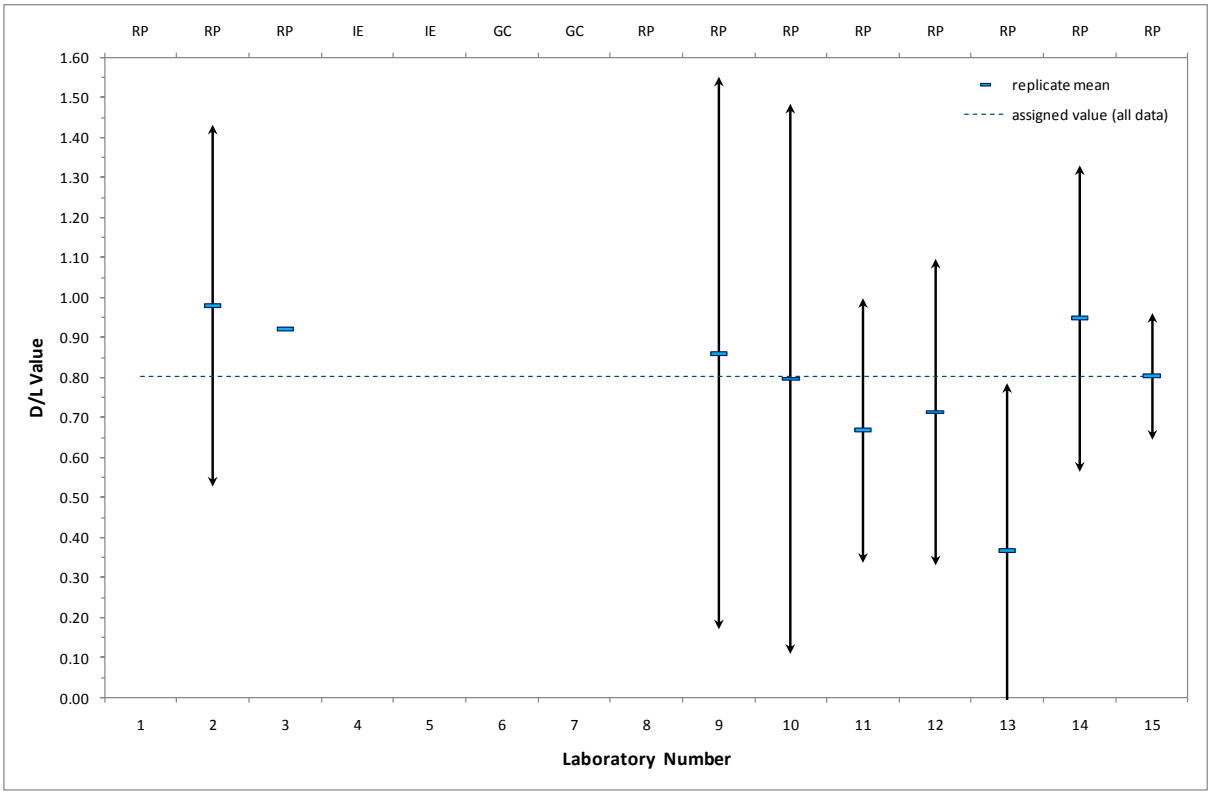


Figure 6.14: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Alanine** D/L Values in Opercula Test Material

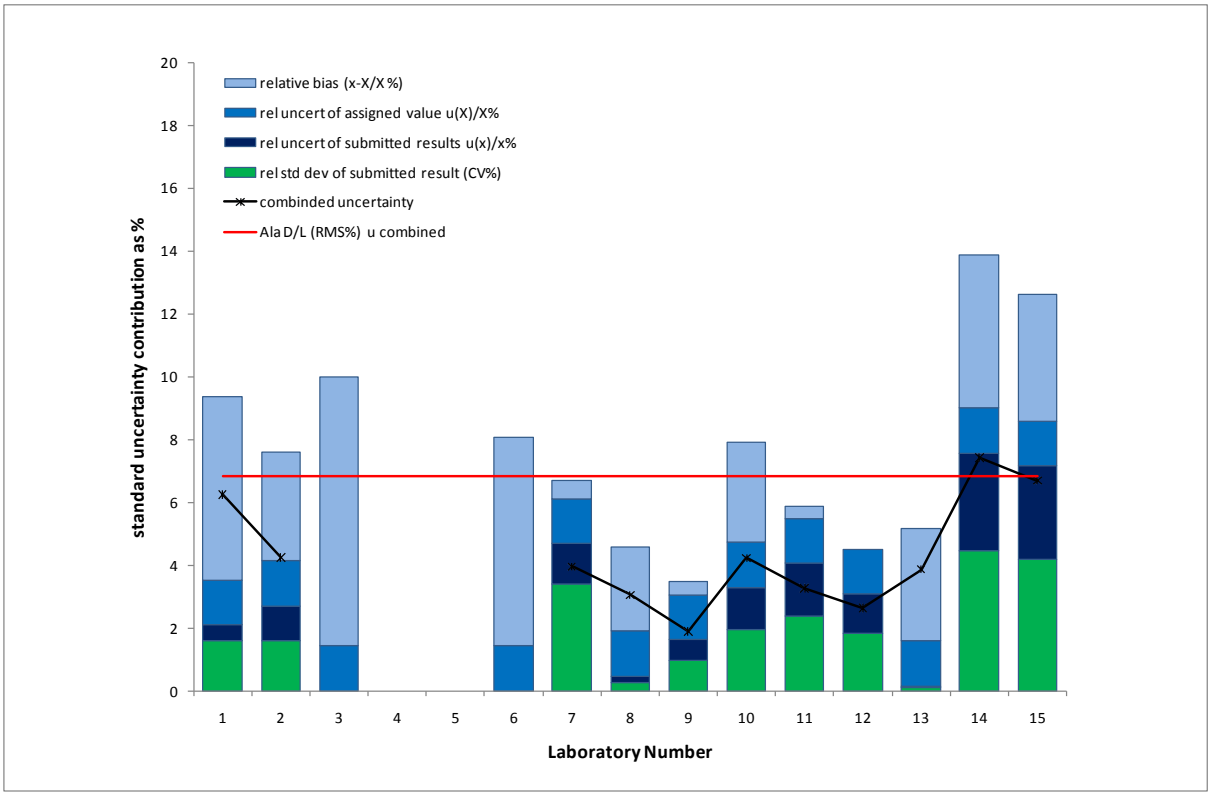


Figure 6.15: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Alanine** D/L Values in Opercula Test Material

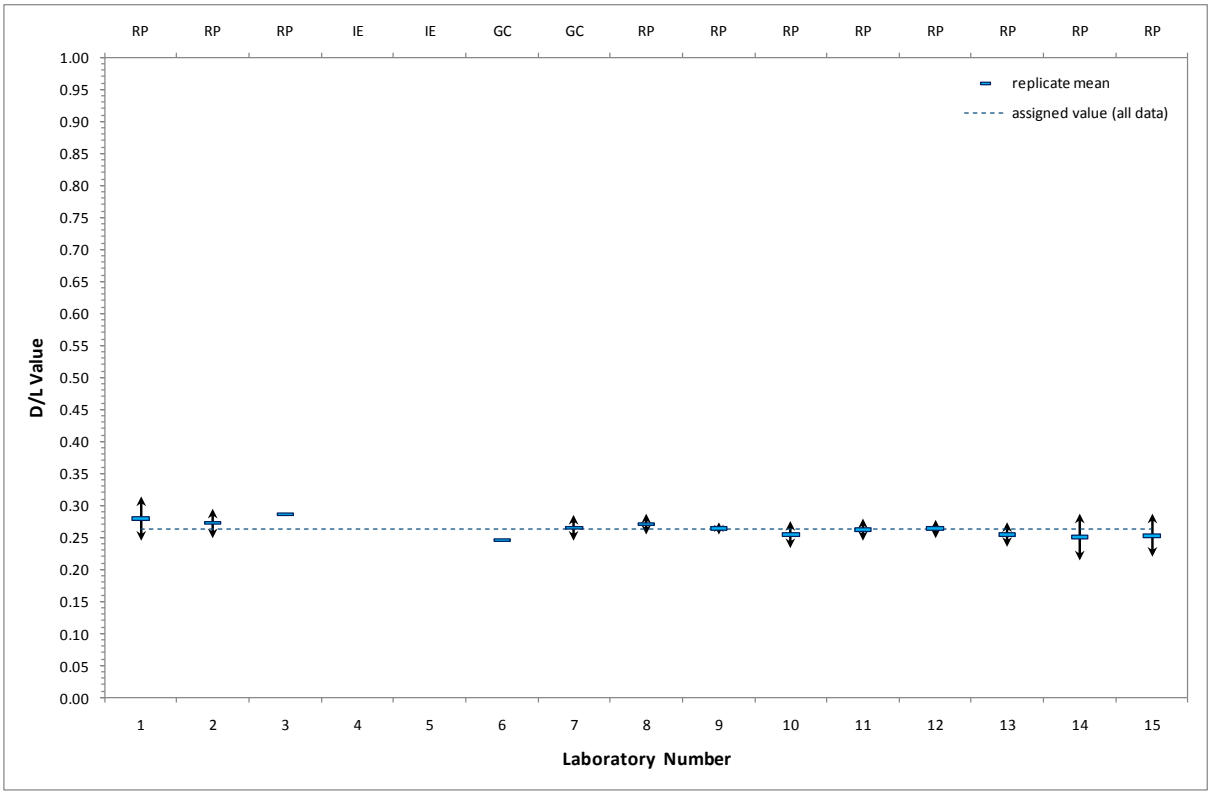


Figure 6.16: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Alanine rpHPLC** D/L Values in Opercula Test Material

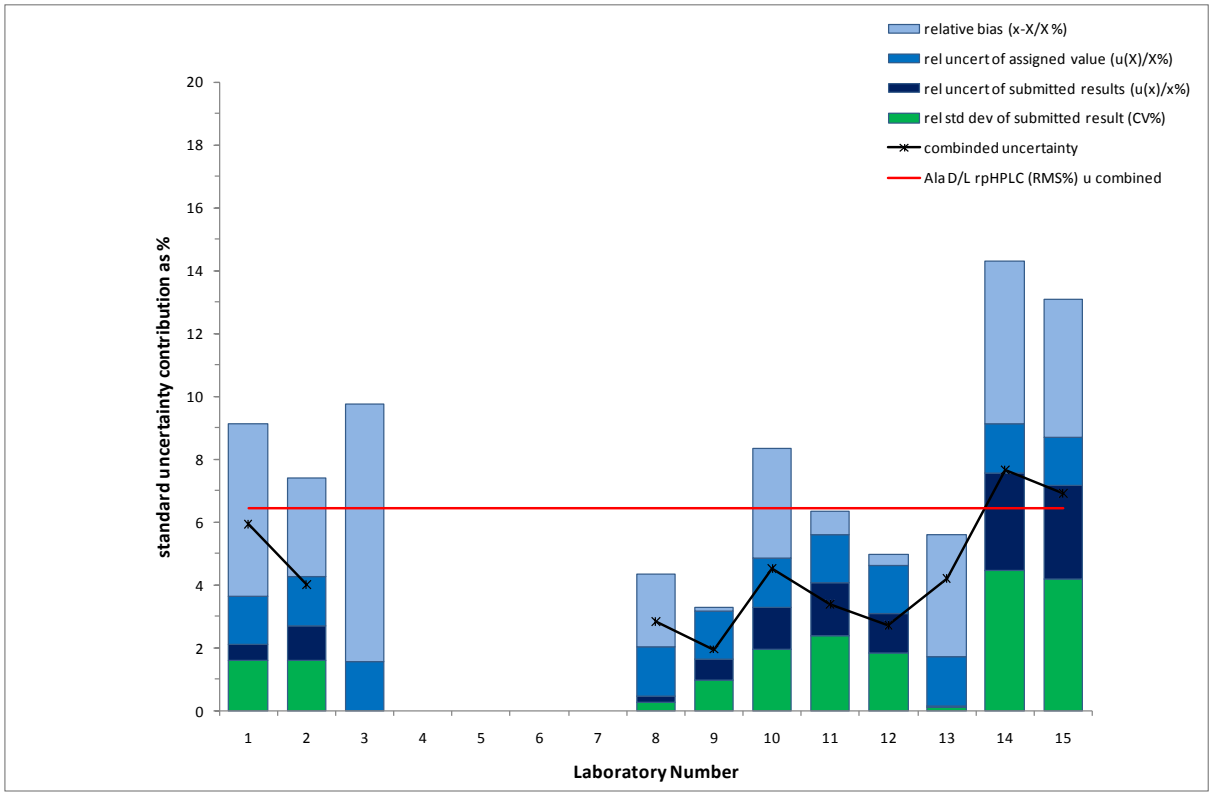


Figure 6.17: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Alanine rpHPLC** D/L Values in Opercula Test Material

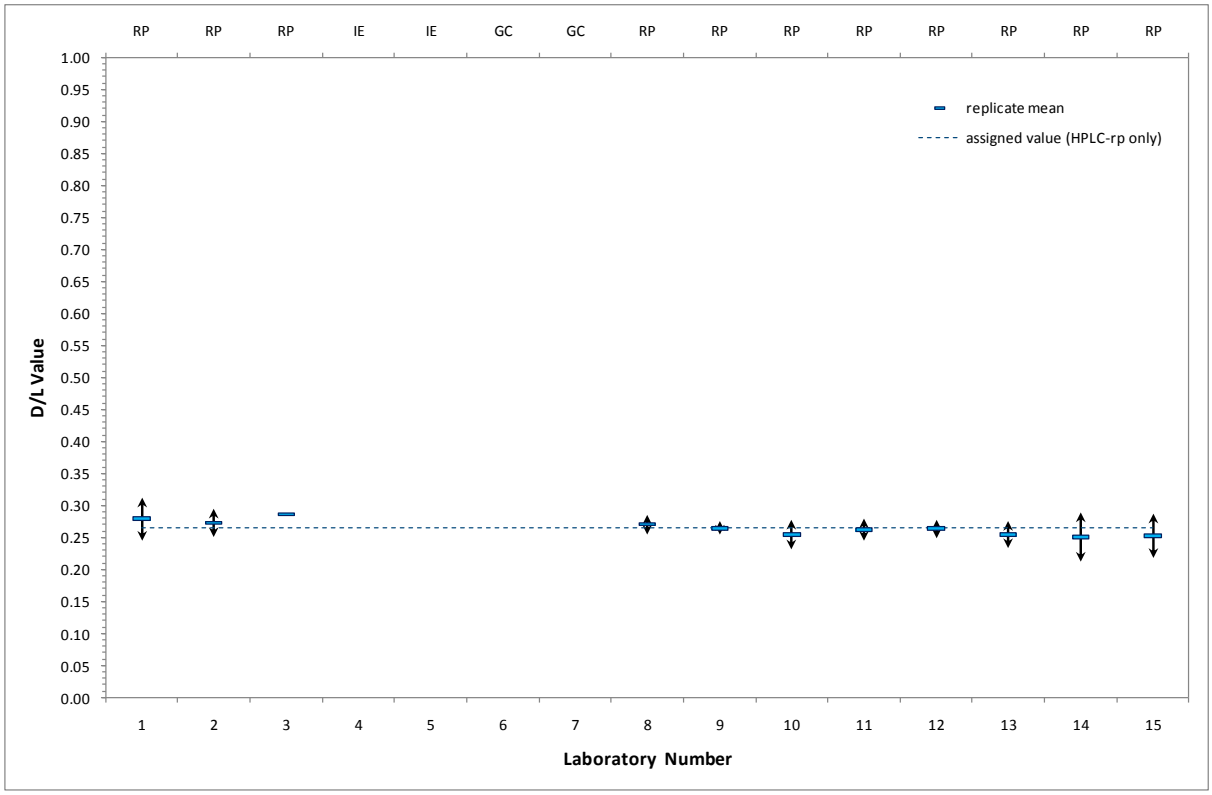


Figure 6.18: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Valine** D/L Values in Opercula Test Material

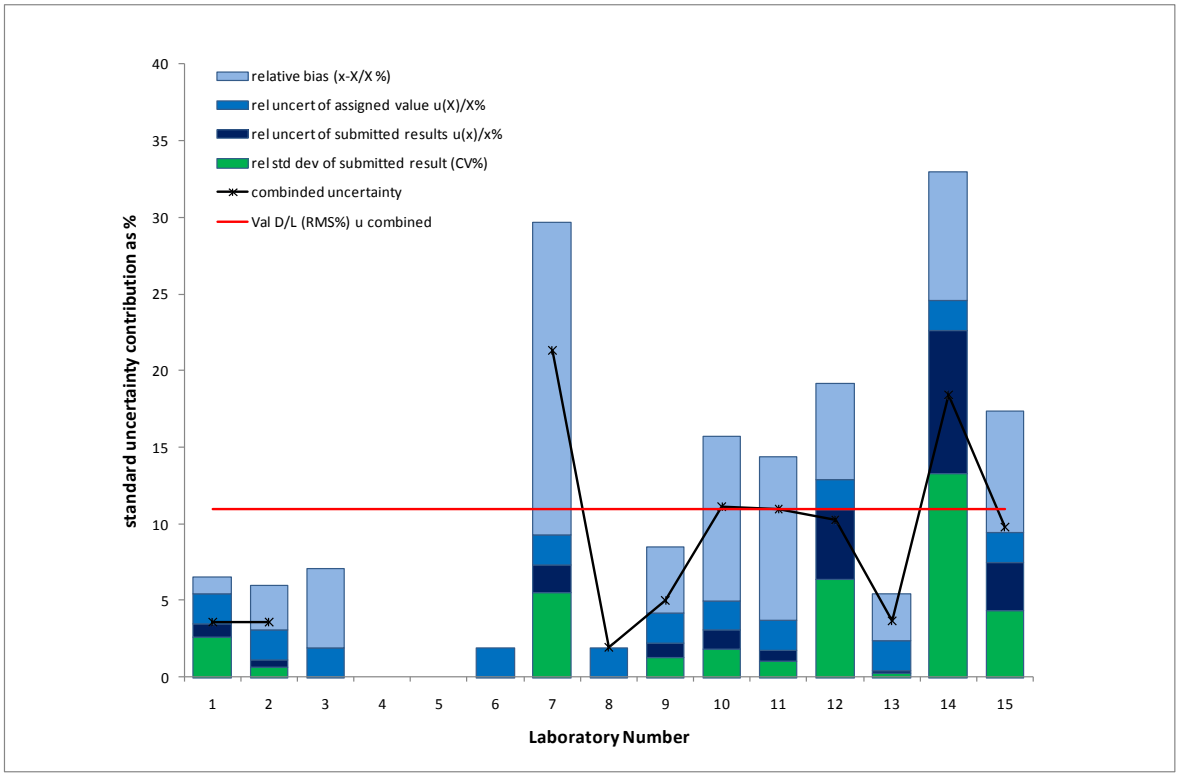


Figure 6.19: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Valine** D/L Values in Opercula Test Material

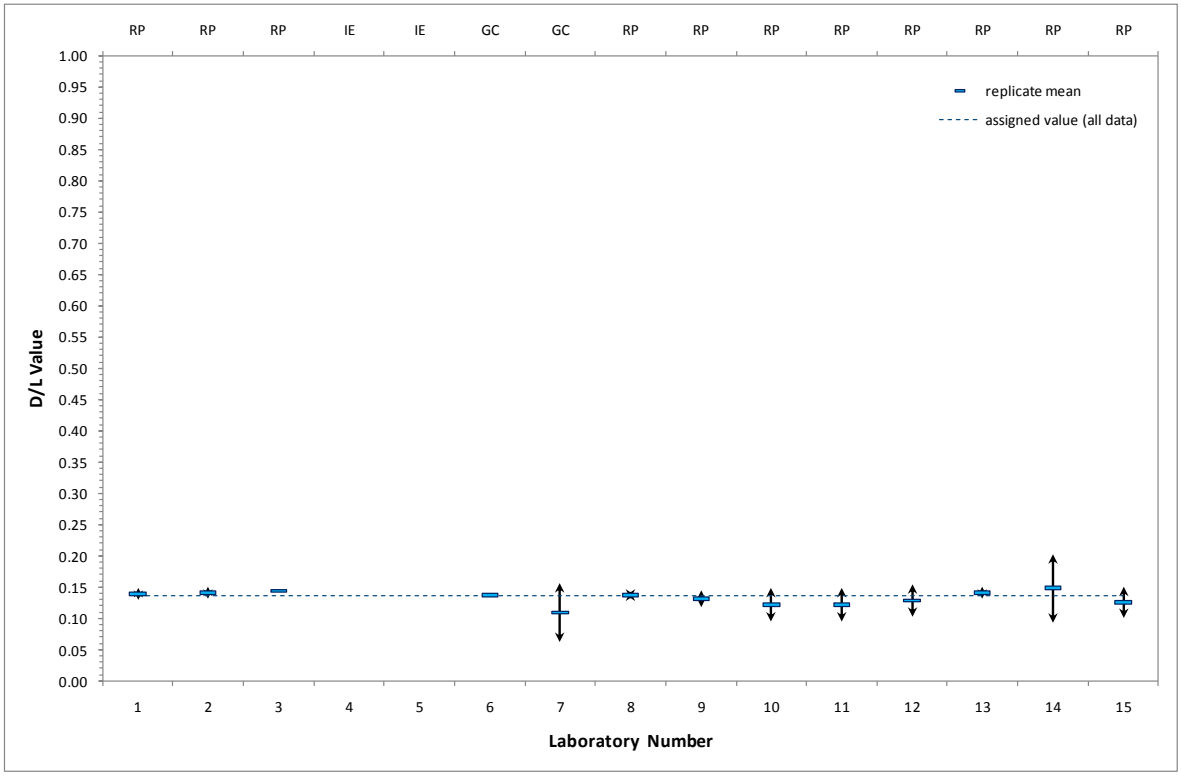


Figure 6.20: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Valine rpHPLC** D/L Values in Opercula Test Material

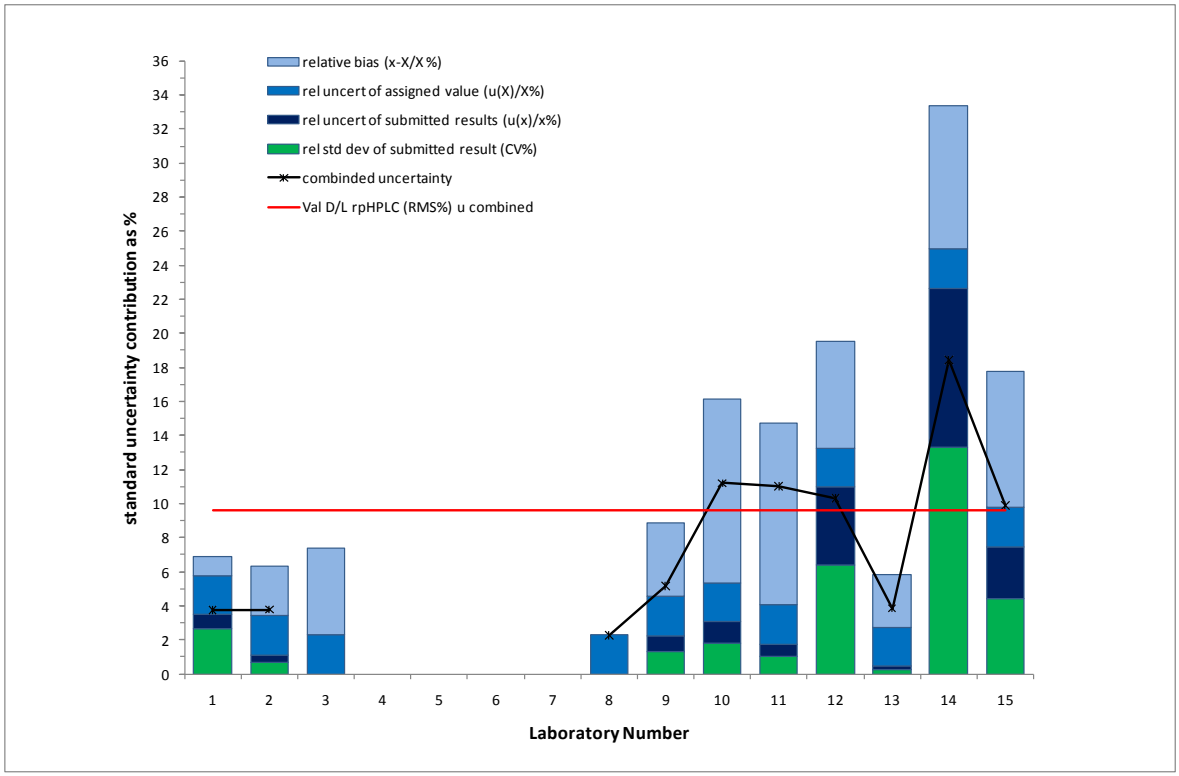


Figure 6.21: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Valine rpHPLC** D/L Values in Opercula Test Material

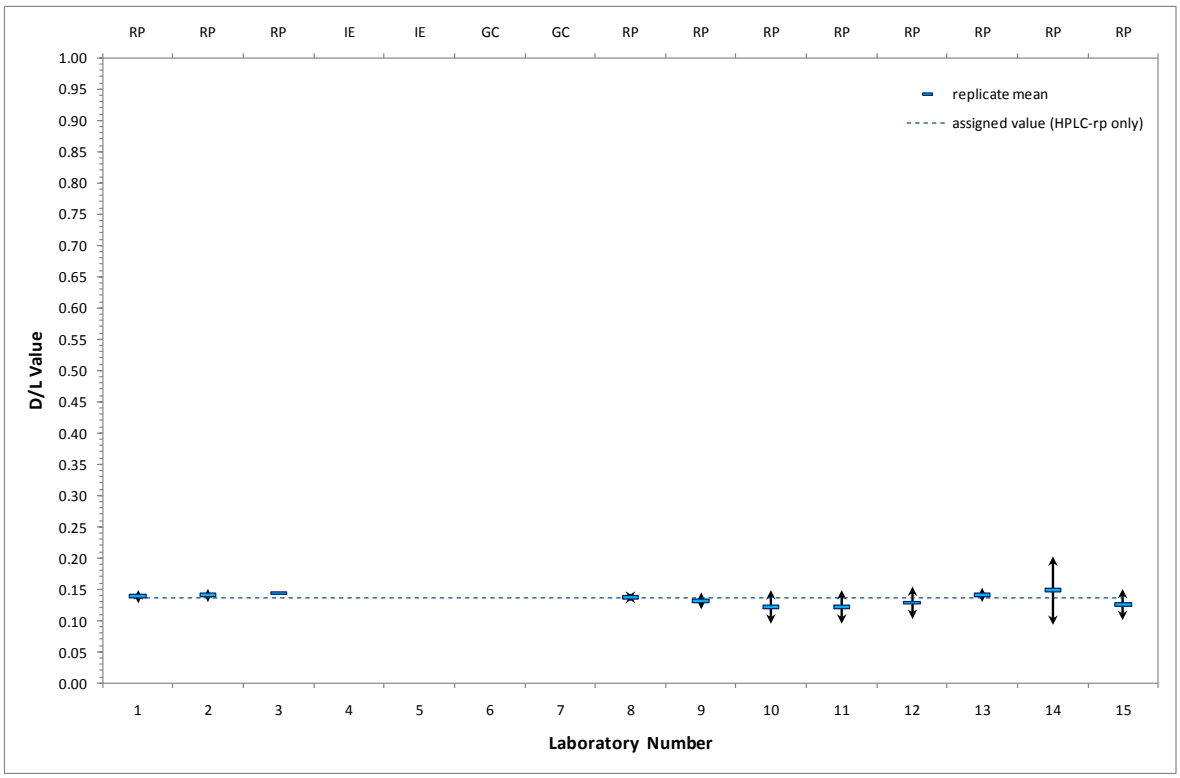


Figure 6.22: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Phenylalanine** D/L Values in Opercula Test Material

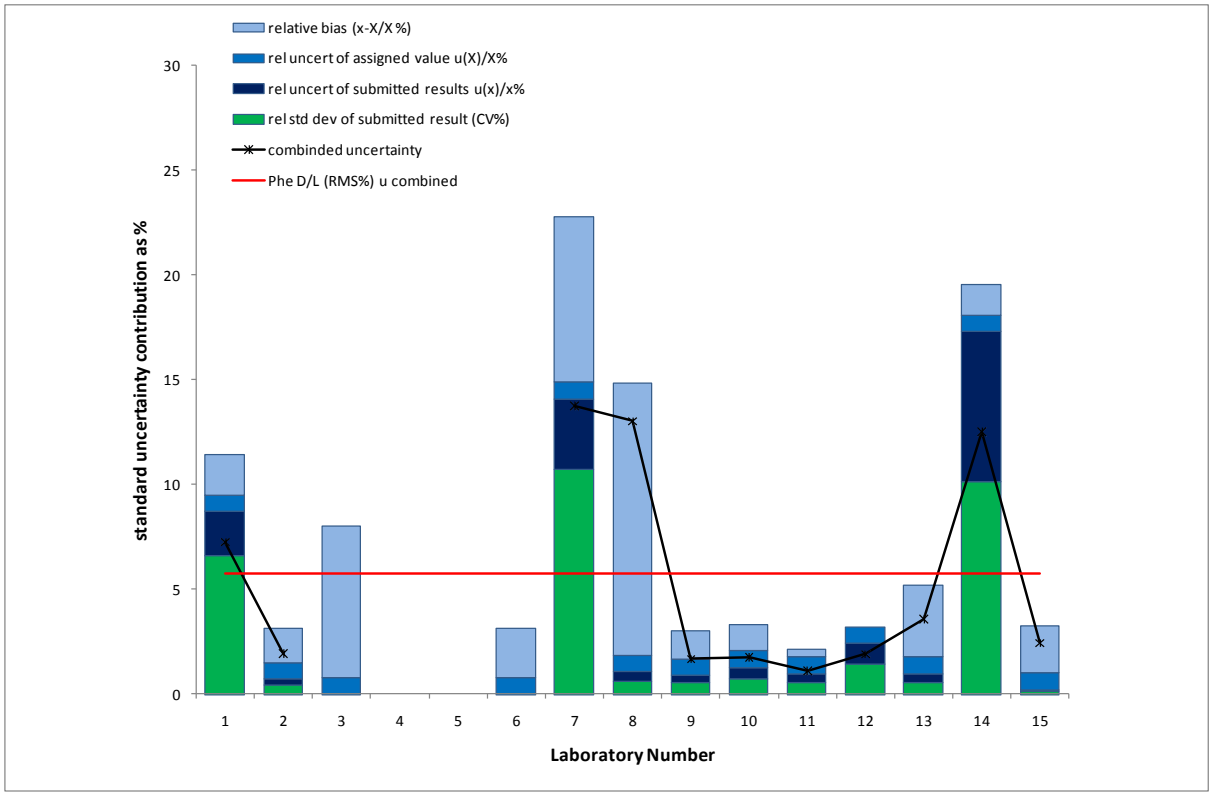


Figure 6.23: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Phenylalanine** D/L Values in Opercula Test Material

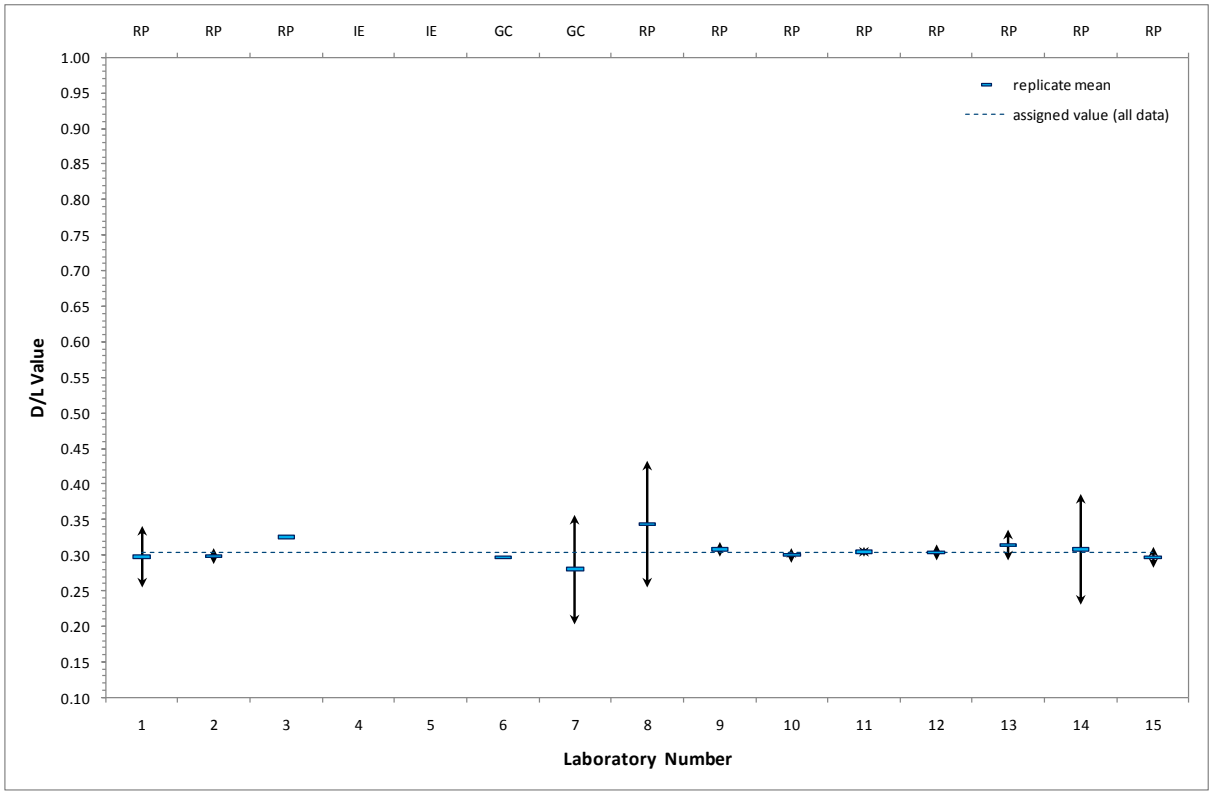


Figure 6.24: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Phenylalanine rpHPLC** D/L Values in Opercula Test Material

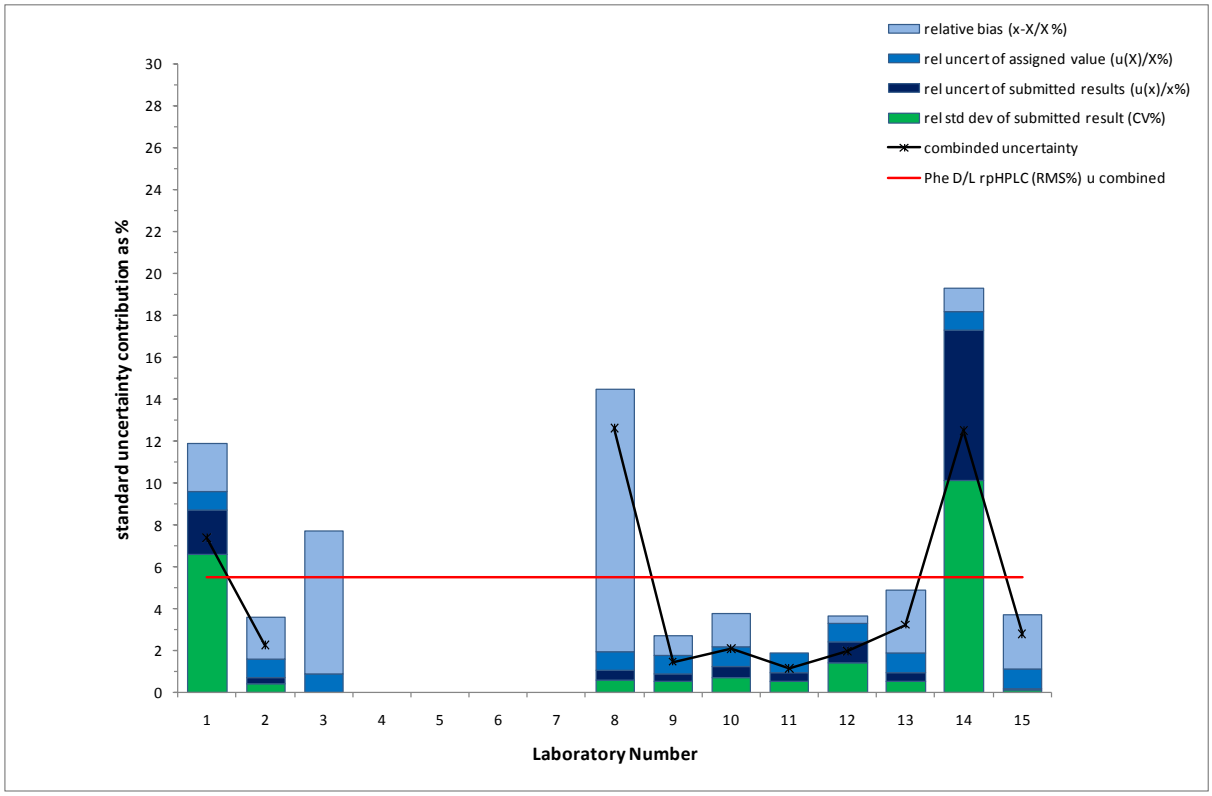


Figure 6.25: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Phenylalanine rpHPLC** D/L Values in Opercula Test Material

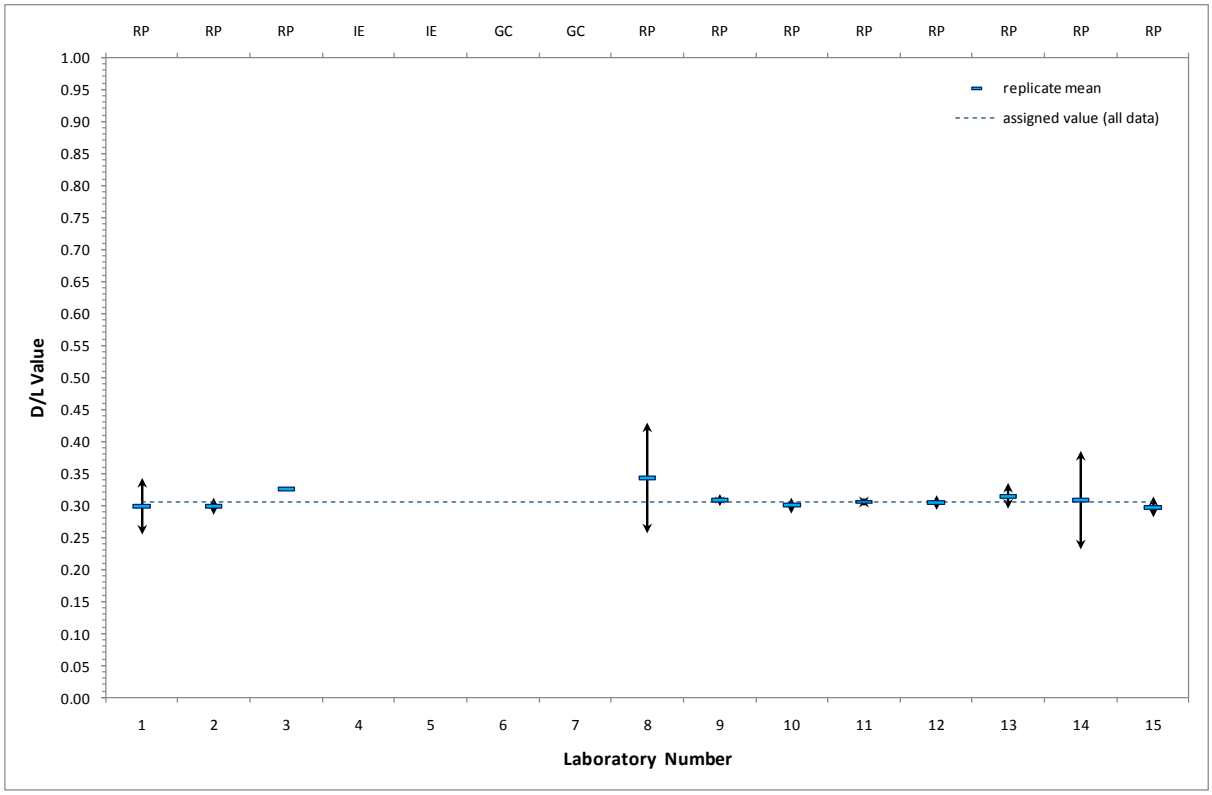


Figure 6.26: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **D-Alloisoleucine/L-Isoleucine** Values in Opercula Test Material

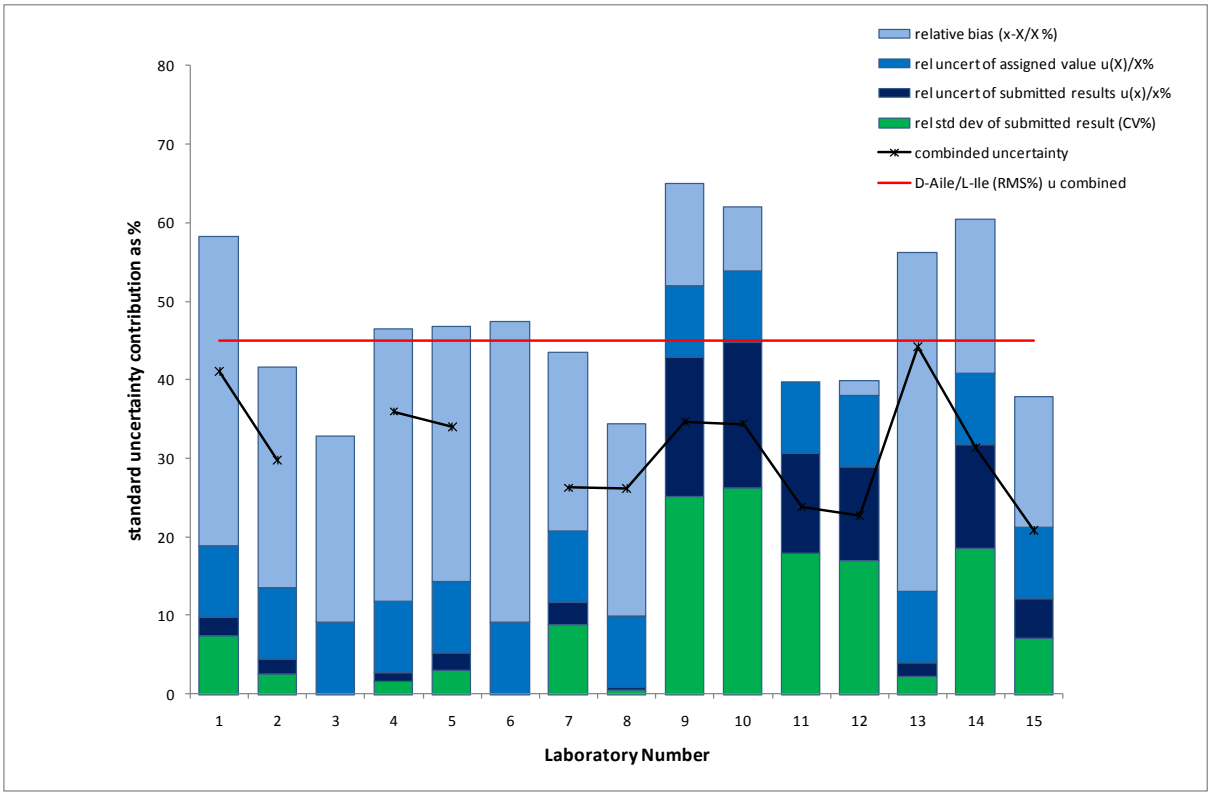


Figure 6.27: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **D-Alloisoleucine/L-Isoleucine** Values in Opercula Test Material

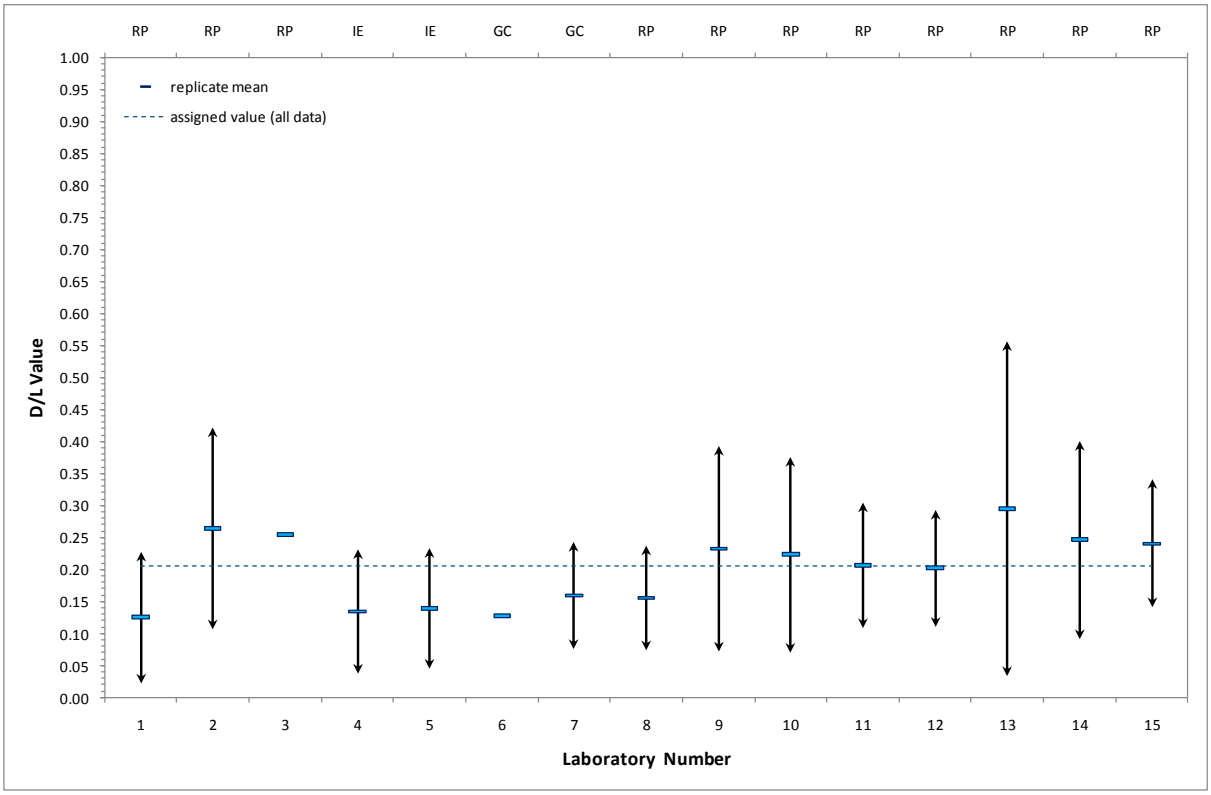


Figure 6.28: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **D-Alloisoleucine/L-Isoleucine rpHPLC** Values in Opercula Test Material

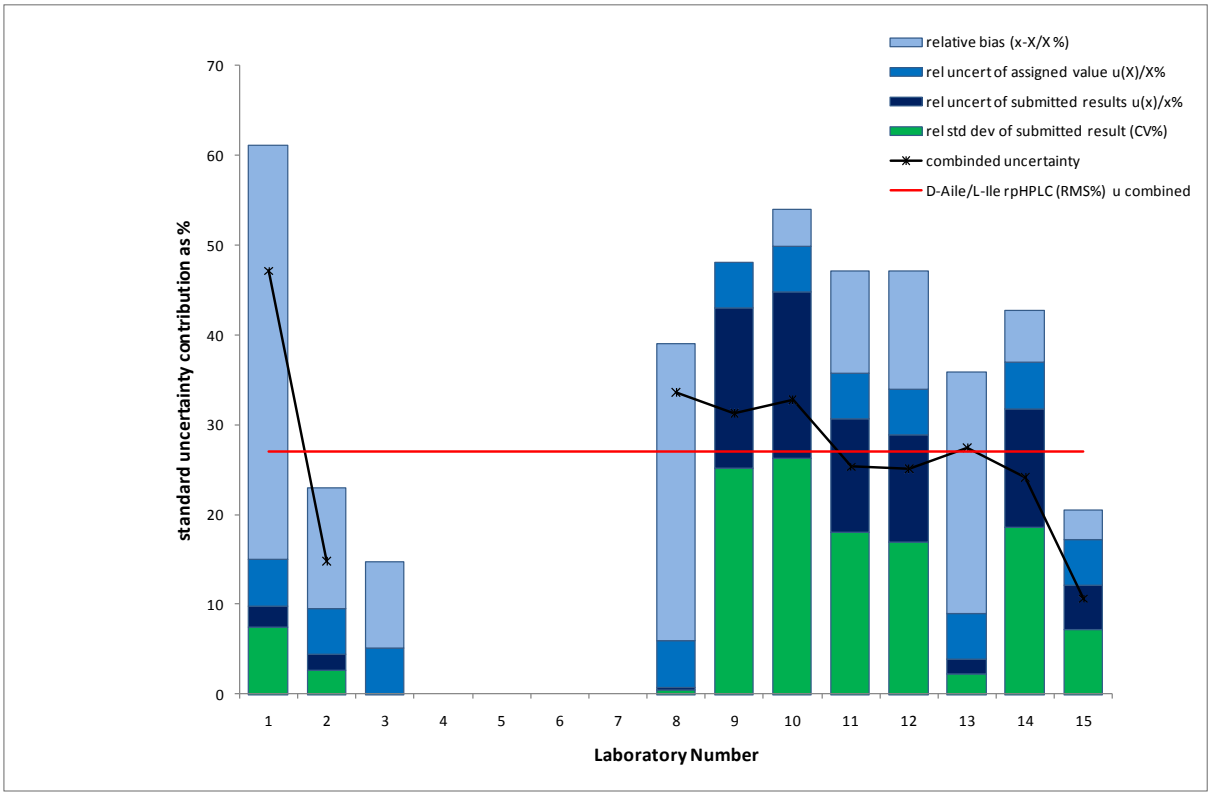


Figure 6.29: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **D-Alloisoleucine/L-Isoleucine rpHPLC** Values in Opercula Test Material

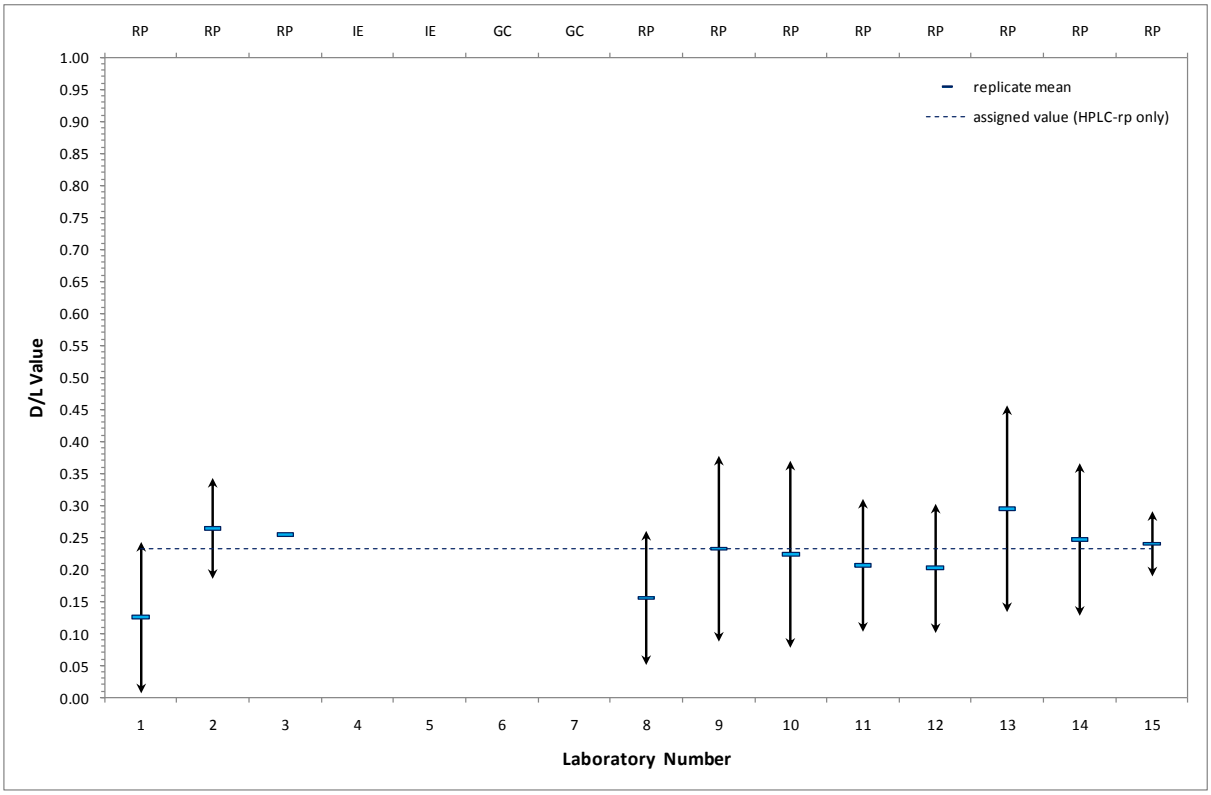


Figure 6.30: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Leucine** D/L Values in Opercula Test Material

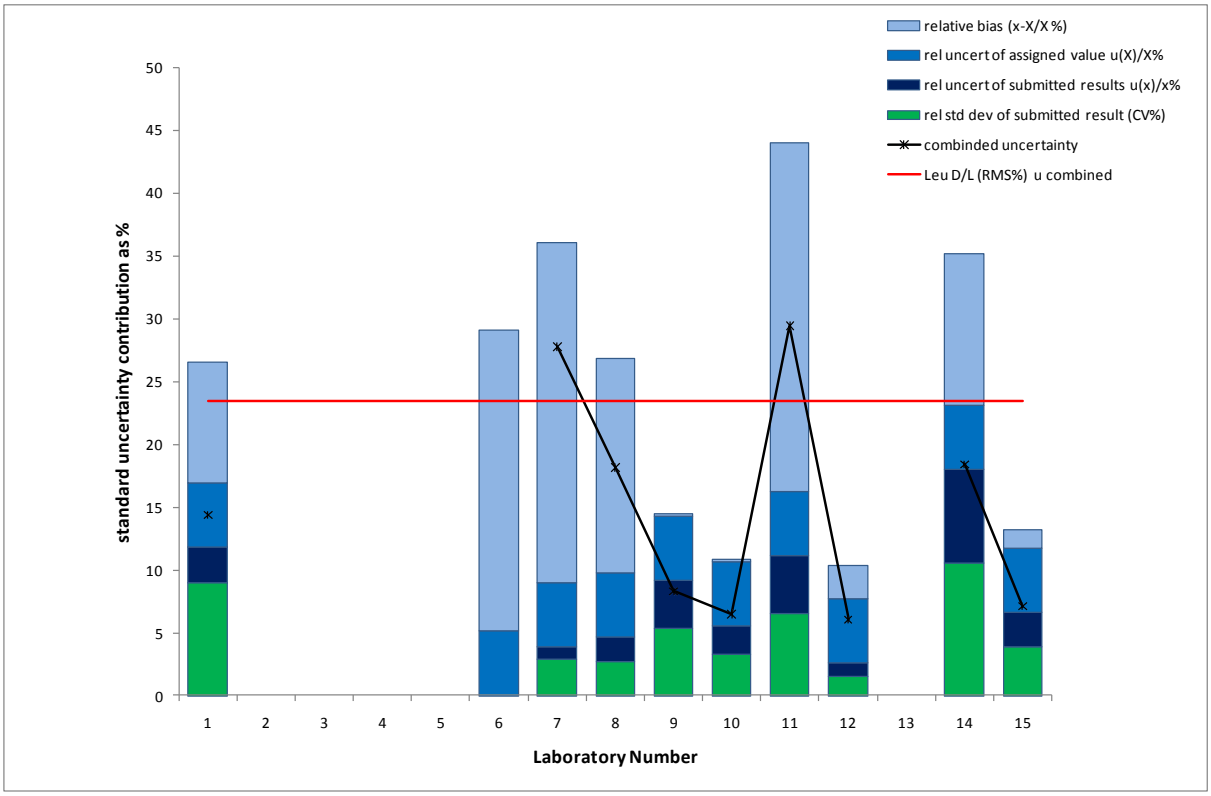


Figure 6.31: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Leucine** D/L Values in Opercula Test Material

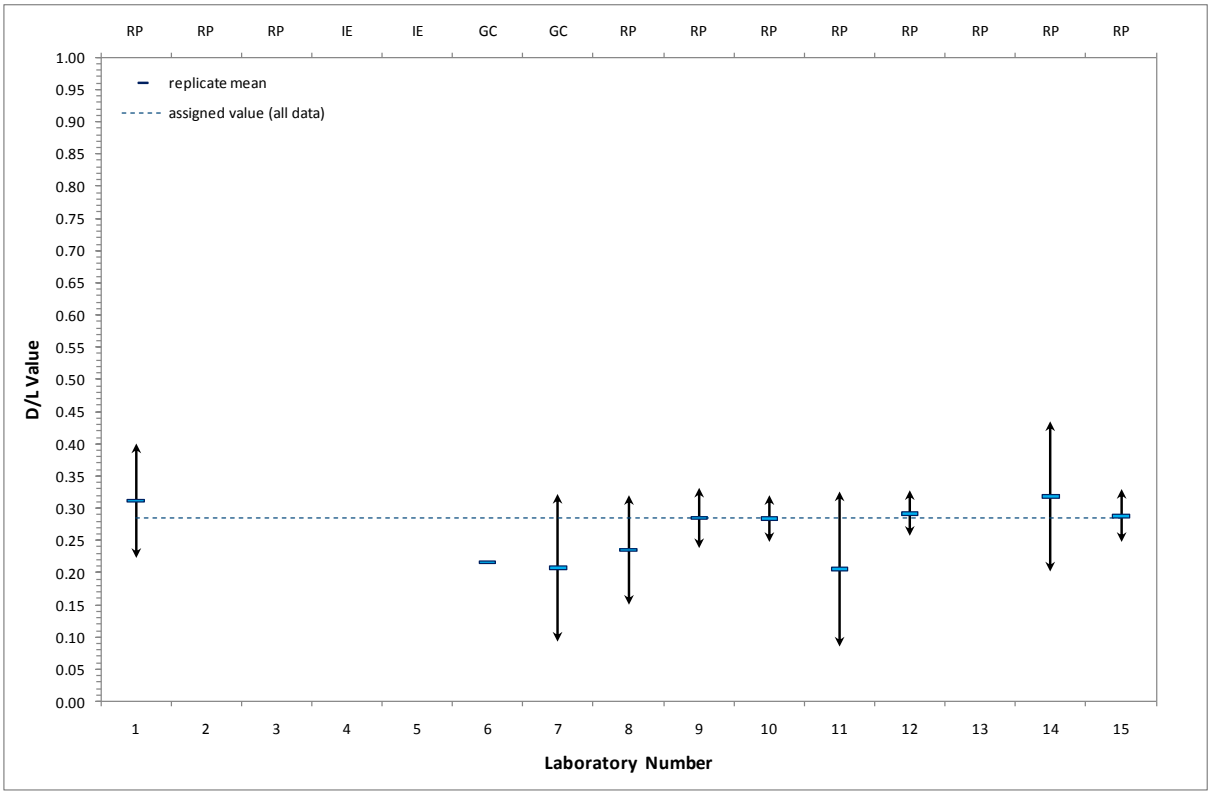


Figure 6.32: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Leucine rpHPLC** D/L Values in Opercula Test Material

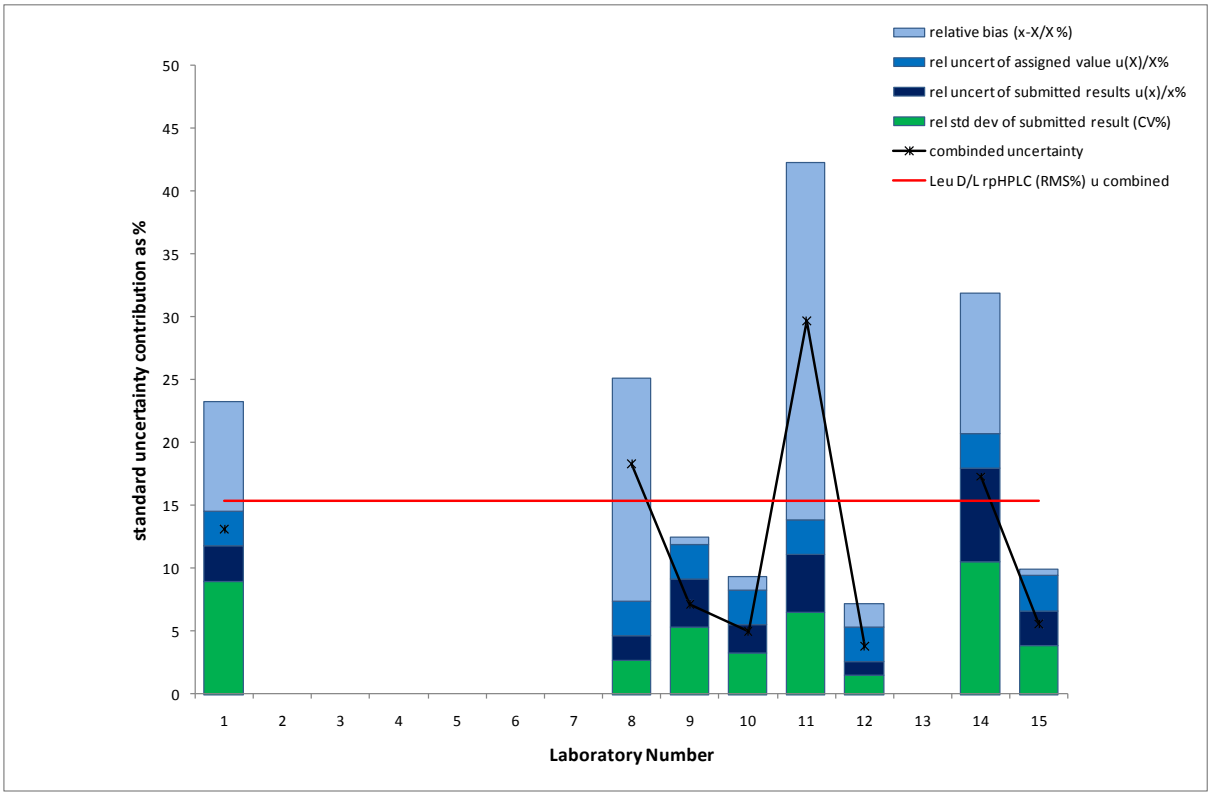


Figure 6.33: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Leucine rpHPLC** D/L Values in Opercula Test Material

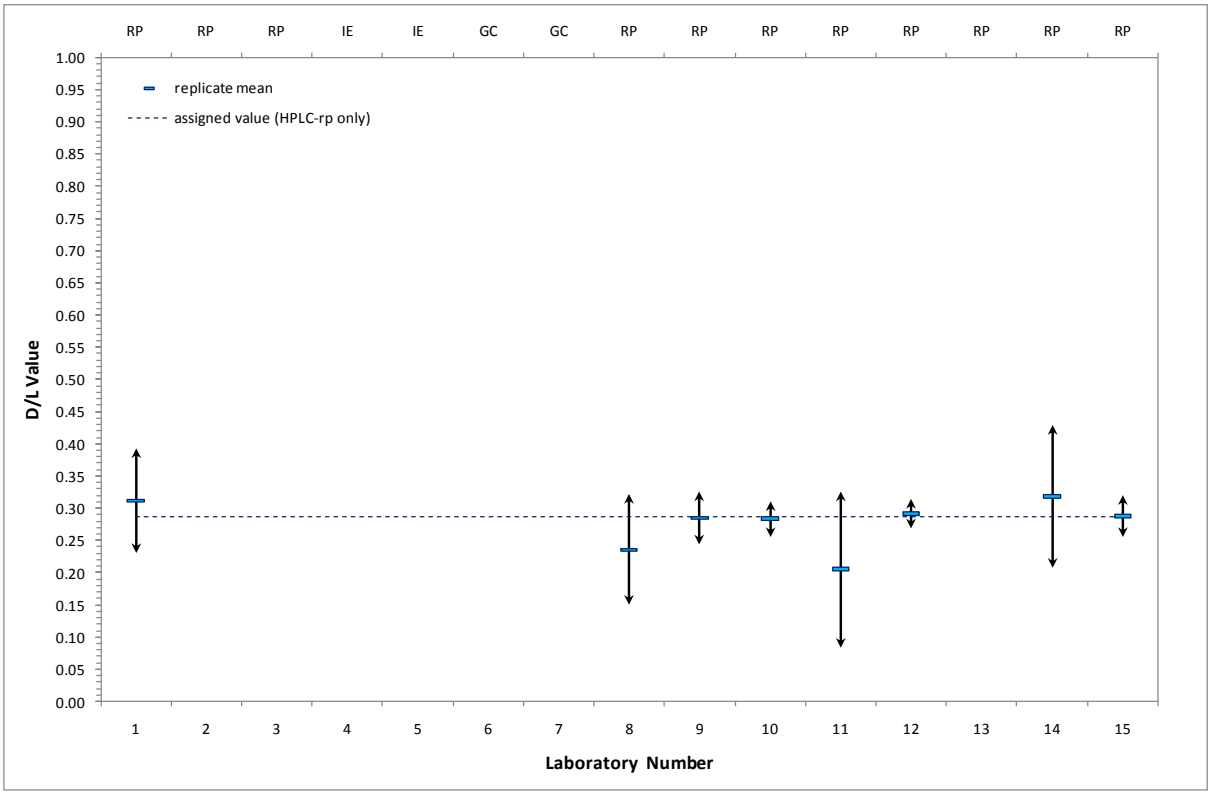


Figure 6.34: Standard Uncertainty Contributions and Combined Uncertainty for each Laboratory against an Estimated Average Combined Uncertainty for **Tyrosine** D/L Values in Opercula Test Material

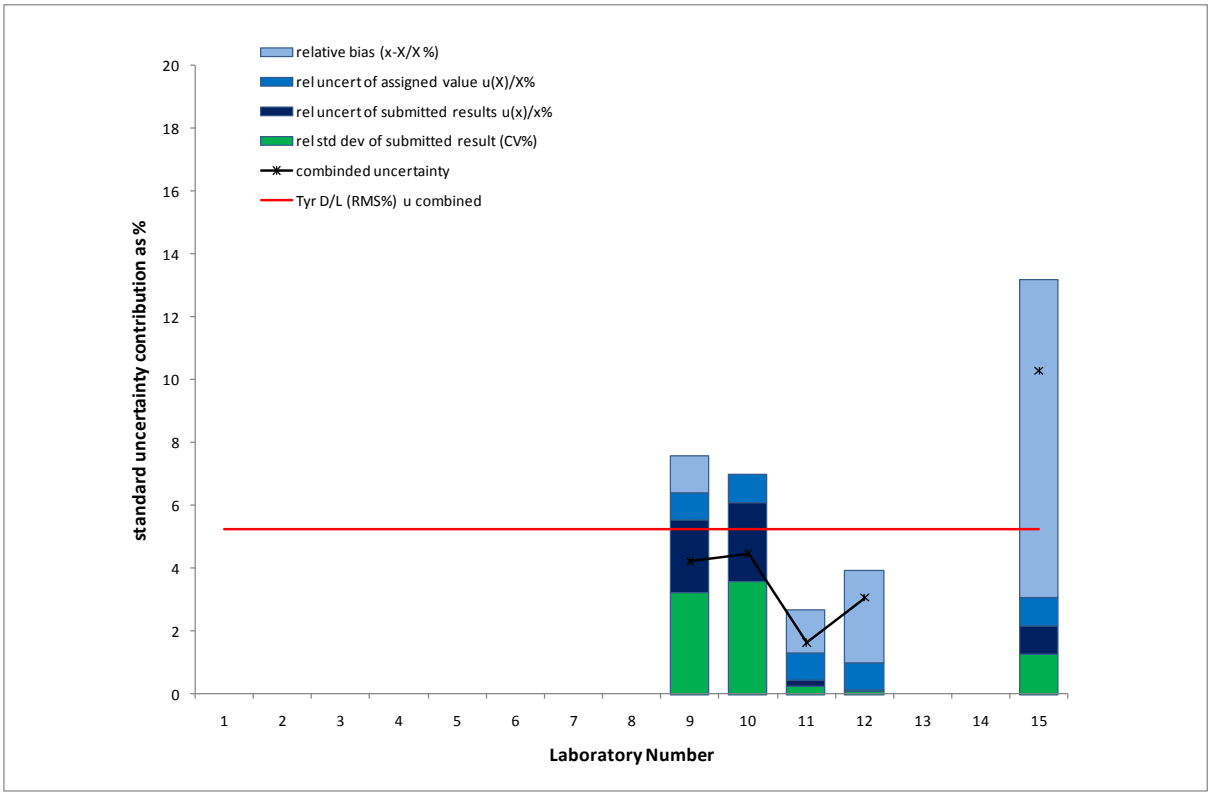
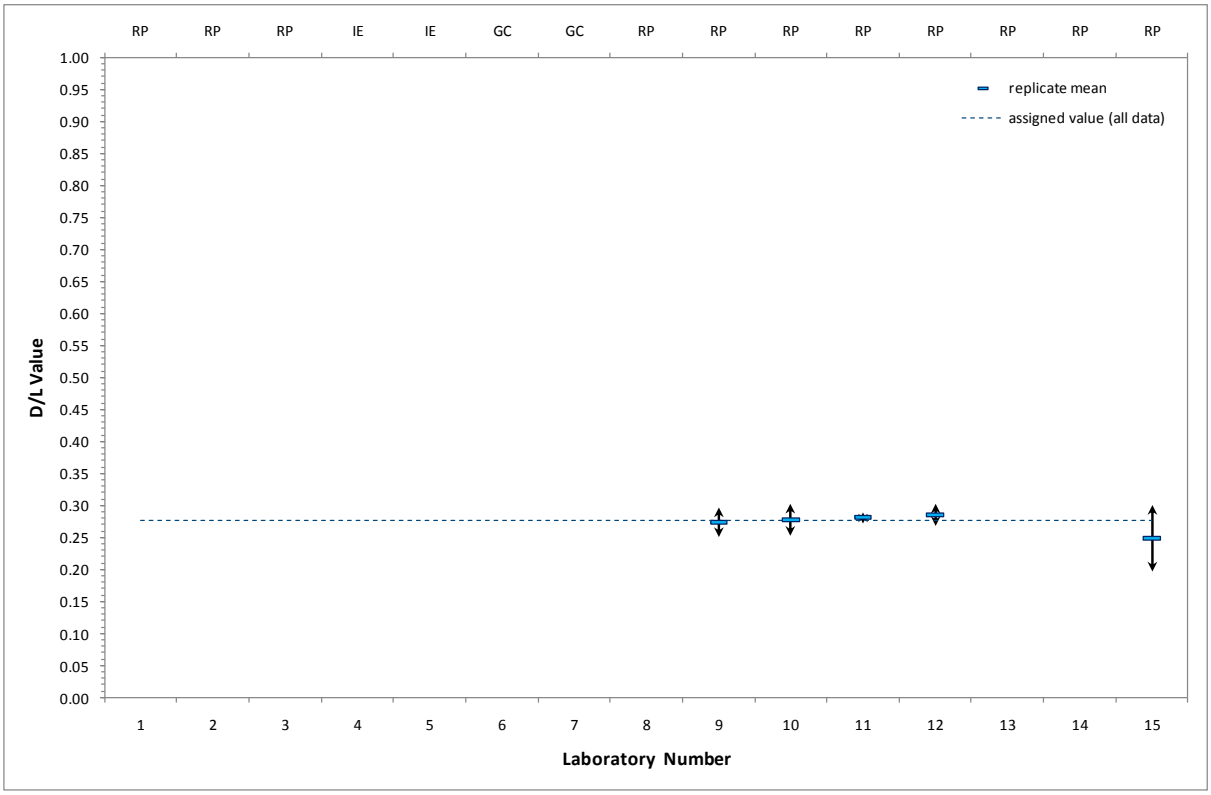


Figure 6.35: Effect of Expanded Uncertainty for each Laboratory at 95% Confidence on **Tyrosine** D/L Values in Opercula Test Material



Appendix 1: Analytical Methods Used by Participants

Reverse Phase HPLC/ HPLC-Ion Exchange

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

THAA's REHYDRATION	
Volume of rehydration fluid added as µl/mg of original sample	
10 µl/mg	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	001
Hypersil BDS	009, 010, 011, 012, 013, 014, 015
Hypersil BDS /5/120/4772	002, 003
Pickering Labs Sodium Cation Exchange	004, 005
Supelcosil LC-18-DB(rp)/6520/5-1452	008
Column Packing:	
Silica	002, 003, 008
Sodium	004, 005
Functional group; C_{18}	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
End capped (Yes)	002, 003, 008
Column width (mm)	
3mm	001, 002, 003, 004, 005
5mm	009, 010, 011, 012, 013, 014, 015
Column length (mm)	
250mm	001, 002, 003, 004, 005, 009, 010, 011, 012, 013, 014, 015
Guard Column not used	
No	001, 002, 003, 004, 005
HPLC Column Temperature (^{o}C):	
25 ^{o}C	001, 009, 010, 011, 012, 013, 014, 015
30 ^{o}C	002, 003, 004, 005, 008
MOBILE PHASE	
Mobile phase programme:	
Gradient	001, 002, 003, 004, 005, 009, 010, 011, 012, 013, 014, 015
Mobile phase components (please state; i.e.; sodium acetate buffer/ methanol/ acetonitrile):	
Sodium acetate Buffer (pH 6.00)	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
Methanol	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
Acetonitrile	001, 002, 003, 008, 009, 010, 011, 012, 013, 014, 015
Sodium citrate buffer (pH 3.12)	004. 005
Sodium citrate buffer (pH 3.86)	004, 005
Sodium chloride buffer (pH 11.5)	004, 005
Sodium acetate Buffer (pH 6.00) Gradient: Starting % | Final %| time (mins) | flow rate (ml/min)	
95%|76.6%|31mins|0.56ml/min	001a
76.6%|46.2%|95min|0.60ml/min	001b
95%|5%|83min|0.500ml/min	002, 003
95%|50%|88min|0.560ml/min	008
95%|46.2%|95min|0.56ml/min	009, 010, 011, 012, 013, 014, 015

MOBILE PHASE continued	
Methanol Gradient: Starting % | Final %| time (mins) | flow rate (ml/min)	
5%|23%|31mins|0.56ml/min	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|0.56mi/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, 007
HYDROLYSIS FOR THAA's	
Sample Weight used for analysis (mg):	
75 - 90 mg	006, 007
Vials used for hydrolysis:	
Glass	006, 007
Acid Used:	
6M HCl	006, 007
Vials flushed with N_2:	
Yes	006, 007
Please give details of any other treatment prior to hydrolysis:	
Comments received (006, 007); Samples weighed into hydrolysis vials without drying; fossil samples are always dried in vacuo prior to weighing for hydrolysis.	
Oven Temperature (^{o}C):	
105 ^{o}C	006, 007
Heating Time (hours):	
22 hrs	006, 007
SAMPLE CLEAN UP / DESALTING	
Was cation exchange resin used?	
No	006, 007
Was HF used to separate amino acids from precipitate?	
Yes	006, 007
Was sample dried prior to Derivatization?:	
Yes	006, 007
Please give details of sample drying conditions:	
Under nitrogen stream Drying Temp; 50 ^{o}C (in heating block) Drying time; 1 hr	006, 007 006, 007 006, 007

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

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

DETECTION	
Detector Type:	
Flame ionisation	006, 007

Comments received (006, 007);

NDP not used for these samples, but used in previous studies – both NPD and FID give same D/L values

ANYTHING ELSE?

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

Comments received (006, 007);

Summary of the preparation sequence:

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

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

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

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

5) Isopropanol esterification – one hour at 105 deg.

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

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

small, 2 or even 3 ul will be injected.

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

Internal Quality Control

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

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

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

Abbreviations

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

Symbols

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

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

Terms and Definitions

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

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

Accuracy

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

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

Analysis of Variance (ANOVA)

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

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

Assigned Value $\hat{X}$

The best estimate of the true value of the measurand.

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

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

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

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

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

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

Bias
estimate of a systematic measurement error (VIM 2.18)

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

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

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

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

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

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.

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

Experimental standard deviation of the mean.

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

External Quality Control (EQC)

See **Quality Control (QC).**

F_1 and F_2

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

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

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

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

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

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

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

(Homogeneity) Analytical Precision (s_{an})

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

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

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

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

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

(Homogeneity) Sampling Precision (s_{sam})

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

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

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

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

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

Homogeneity Target standard deviation (σ_h).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

standard deviation from a collaborative trial as their own **standard uncertainty** estimate.

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

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

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

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

Standard Deviation (s, sd or σ)

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

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

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

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

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

Standard Error of the Mean.

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

Standard Uncertainty ($u(x)$)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

$u(\bar{x}) = {s_{\bar{x}}}/{\sqrt{n}}$ where $s_{\bar{x}}$ = the std dev of replicate values
and n = the number of replicate values submitted

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

Submitted Result or Value (x *or* x_i)

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

Systematic Error

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

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

Target Standard Deviation (σ_p)

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

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

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

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

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

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

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

Trueness

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

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

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

t-value

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

Such that, for a 95% confidence interval; $$CI = \bar{x} \pm \left[t_{(2,o.o5,df)} \times \frac{\sigma}{\sqrt{n}}\right]$$

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

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

Uncertainty of Measurement / Measurement Uncertainty (MU)

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

Welch-Satterthwaite formula

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

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

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

z-Score

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

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

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)

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