

Uncertainty of Measurement Calculation for the Abbott Murex HCV EIA using the Guide to the Expression of Uncertainty in Measurement (GUM) Approach

Detailed discussion

Version 2.0

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Written for the

Serology Uncertainty of Measurement Working Party

1. Introduction:

Estimations of Uncertainty of Measurement (MU) for serological assays using the ISO “Guide to the Expression of Uncertainty in Measurement” (GUM) are unavailable in the literature. There are some similarities between serological assays and clinical chemistry assays as many of these tests are performed on the same instruments. Clinical chemistry has often used the results of quality control (QC) to estimate MU [1]. However, a similar approach to estimating MU of serological assays need validation. A single assay, Abbott Murex anti-HCV antibody (HCV) enzyme immunoassay (EIA) was selected as a representative model for microtitre plate EIAs because this assay was used by many laboratories throughout Australia and significant information regarding this assay was available.

2. Goal:

To estimate MU of the Abbott Murex HCV EIA when the assay was performed according to the manufacturer’s instructions.

To compare the estimated MU results with the QC testing results obtained from data collected from 18 laboratories over approximately one year

3. Step 1. Measurement procedure:

The measurement of HCV is achieved using an indirect immunoassay procedure [2]. The steps can be summarised as in Table 1.

Table 1: Summary of the Abbott Murex HCV EIA procedure including the volume of reagents, time and temperature of incubation and reading requirements of the assay.

Step	Details
Reconstitution of conjugate	20mL of buffer added by pouring into concentrate
Reconstitution of substrate	35mL of solution added by pouring into concentrate
Sample addition	20µL sample
Diluent addition	180µL diluent
Incubation	60 minutes (65±5min); 37°C (37±1 °C)
Wash	NA
Conjugate addition	100µL conjugate
Incubation	30 minutes (32.5±2.5min); 37°C (37±1 °C)
Wash	NA
Substrate addition	100µL substrate
Incubation	30 minutes (32.5±2.5min); 37°C (37±1 °C)
Stop solution addition	50µL substrate
Blank	Read on air
Read at 450 nm	Read at 450 nm
Read at 620 nm	Read at 620nm

3.1 Component measured

The **analyte** was the anti-HCV antibodies present in a serum or plasma sample. The **measurand** was the reactivity of anti-HCV antibodies attaching to specific antigens coated on the solid phase (microtitre plates).

The measurand was calculated as a signal to cut-off ratio (S/Co) using the formula [2]:

$$HCV\ S/Co = \left(\frac{\text{Optical density of test result}}{\text{Mean optical density of negative control} + 0.6} \right)$$

4. Step 2. Identification of the sources of uncertainty

The sources of uncertainty can be grouped as elements contributing to variation:

- Time (T):

Each incubation step has an acceptable period of time specified by the manufacturer. The incubation time is controlled by a laboratory timer that should be calibrated at least every 12 months. The assay manufacturer's instructions for incubation must be adhered to in order to ensure a valid result.

- Temperature (Temp):

Each incubation step requires a temperature-controlled environment. The manufacturer specifies an allowable temperature range for each step. A limit for ambient temperature is not included in the instructions. Reagents must be warmed to 18 – 30 °C before use.

- Volume (Vol):

All sample and reagent addition steps are performed using calibrated pipettes. Variation in the dispense volumes may contribute to imprecise or inaccurate results. The amount of wash buffer used for each wash cycle is also a possible source of variation.

- Reading (Rd):

The amount of measurand in a test sample corresponds with the colour change associated with the assay. The colour change is read 450 nm and 620 nm after the spectrophotometer is blanked on air.

- Operator:

Each operator can introduce minor changes to the procedure. These changes may be random or systematic and may have a variable effect on the test result.

- Reagent Batch:

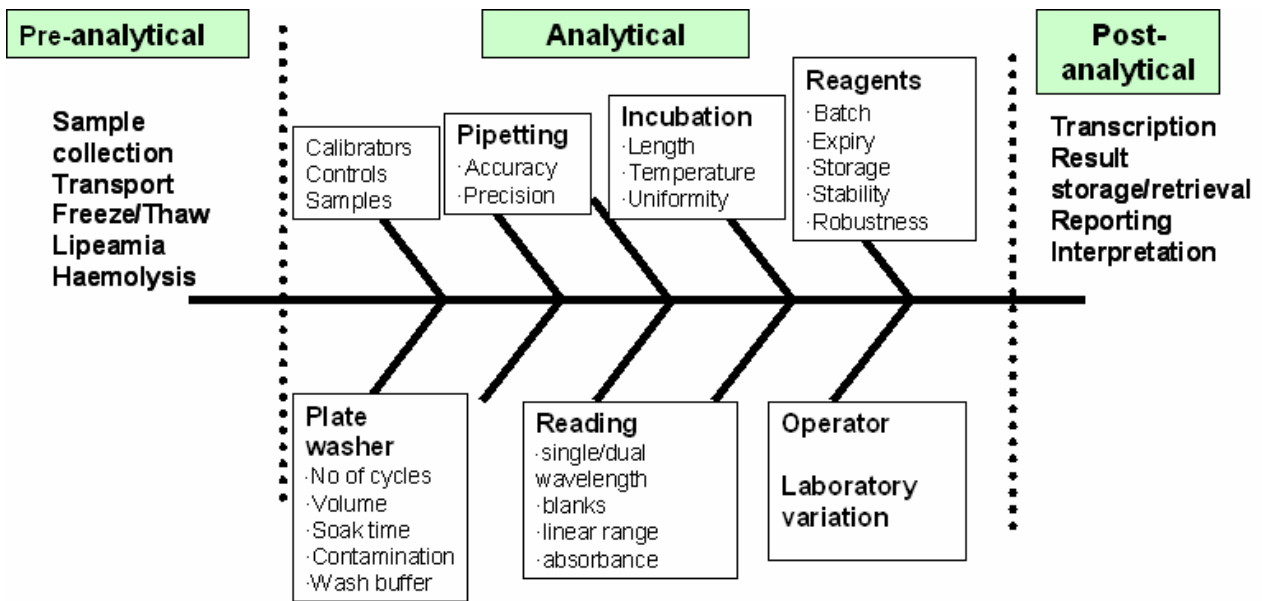
The manufacturing process of each lot number of reagents is known to contribute to variation in the test results. A test kit is comprised of many source components such as antibodies, antigens, buffers and plastics. Although each lot number of the test kits is optimised to standardise lot-to-lot performance, variation in reactivity is experienced.

- Bias:

Differences in results are obtained when a single sample is tested in different laboratories. This may be due to the systemic variation contributed by the sources of variation detailed above. The bias can be defined as the difference between the results produced by a laboratory and the “True Value”. Defining a “True Value” for a polyclonal serological assay is problematic.

The sources of uncertainty can be summarised using the “fishbone diagram” in Figure 1.

Figure 1. A “fishbone diagram” representation of the major sources contributing to variation in the analytical and pre- and post-analytical stages of testing using the Abbott Murex HCV EIA.



Staff training and adherence to Standard Operating Procedures

The pre- and post-analytical stages of the testing process have been excluded from these calculations. It was deemed that the variation introduced by these stages were outside the scope of the exercise and many of the variables are controlled by standard operating procedures, manufacturer’s instructions and validation procedures used by the laboratory. In addition, biological variation was also excluded. Although biological variation may contribute to uncertainty of measurement, appropriate data and understanding of the nature of the effect of variation due to this factor, is lacking. If, or when, additional information regarding the effect of biological variation on serological testing is available, this factor will be considered.

5. Step 3. Quantification of the uncertainty components

5.1. Time

There are three steps where time is critical. Two of these incubation times are expressed as 30 minutes in the package insert but allows +5 minutes. For the purposes of the calculation and in line with the package insert, the time is expressed as 32.5±2.5 minutes. The uncertainty associated with the time taken after the addition of the stop solution and

before the reading of the reaction was considered to be inconsequential as the reaction is stopped at this stage. Assuming the maximum allowable variation of each step as specified by the manufacturer, the uncertainties are shown below in Table 2.

Footnote: If limits of $x \pm a$ (allowable variation) are given without a confidence limit and there is reason to expect that extreme values are likely, it is normally appropriate to assume a rectangular distribution, with a standard deviation (SD) of a /square root of 3. (EURACHEM / CITAC Guide - 8.1.4.) Given that we are assuming the maximum allowable variation it is appropriate to use this calculation to estimate the standard uncertainty. [3]

Table 2. Estimation of the contribution of time to the uncertainty of measurement in each of the major steps of the Abbott Murex HCV EIA procedure.

Description (abbreviation)	Value (x)	Allowable variation (a)	Standard Uncertainty $u(x)$	Relative Standard Uncertainty (RSD)
Unit/calculation	min	\pm min	$\frac{a}{\sqrt{3}}$	$\frac{u(x)}{x}$
Sample/control incubation $T_{s\&c}$	65	5	2.886751346	0.044411559
Conjugate incubation T_{conj}	32.5	2.5	1.443375673	0.044411559
Substrate incubation T_{sub}	32.5	2.5	1.443375673	0.044411559
Stop solution	NA			

Using EURACHEM / CITAC Guide Rule 2 (8.2.6.): For models involving only product or a quotient of quantities e.g. $y = (p \times q \times r \times \dots)$, the combined relative standard uncertainty $u(y)$ is given by:

$$u(y[p, q, r, \dots]) = \sqrt{\left(\frac{u(p)}{p}\right)^2 + \left(\frac{u(q)}{q}\right)^2 + \left(\frac{u(r)}{r}\right)^2 + \dots}$$

The total RSD of time (RSD_T) was calculated by combining the RSD of each component of time.

	$u(T_{s\&c})$	$u(T_{conj})$	$u(T_{sub})$	Total
RSD	0.044411559	0.044411559	0.044411559	
RSD^2	0.001972387	0.001972387	0.001972387	
$\sum RSD^2$				0.00591716
$\sqrt{\sum RSD^2}$				0.07692308

Combined Relative Standard Uncertainty of time (RSD_T) = 0.07692308

5.2. Temperature

There are three steps where temperature was critical. Again, the temperature at and after the addition of stop solution and before the reading of the reaction was deemed to be inconsequential. Assuming the maximum allowable variation of each step as specified by the manufacturer and using the formulae described above, the uncertainties are shown below in Table 3.

Table 3. Estimation of the contribution of temperature to the uncertainty of measurement in each of the major steps of the Abbott Murex HCV EIA procedure.

Description (abbreviation)	Value (x)	Allowable variation (a)	Standard Uncertainty $u(x)$	Relative Standard Uncertainty (RSD)
Unit/calculation	$^{\circ}\text{C}$	$\pm^{\circ}\text{C}$	$\frac{a}{\sqrt{3}}$	$\frac{u(x)}{x}$
Sample and control incubation $Temp_{s\&c}$	37	1	0.577350269	0.015604061
Conjugate incubation $Temp_{conj}$	37	1	0.577350269	0.015604061
Substrate incubation $Temp_{sub}$	37	1	0.577350269	0.015604061
Stop solution	NA			

The total RSD of temperature (RSD_{Temp}) was calculated by combining the RSD of each component of temperature.

	$u(Temp_{s\&c})$	$u(Temp_{conj})$	$u(Temp_{sub})$	Total
RSD	0.015604061	0.015604061	0.015604061	
RSD^2	0.000243487	0.000243487	0.000243487	
$\sum RSD^2$				0.00073046
$\sqrt{\sum RSD^2}$				0.02702703

Combined Relative Standard Uncertainty of temperature (RSD_{temp}) = 0.02702703

5.3. Volume

There are several steps that require the accurate dispensing of a volume of reagent. These steps are summarised below in Table 4. The reconstitution of the conjugate and substrates was considered inconsequential, as the manufacturer supplied the buffer in pre-measured volumes. The standard uncertainty $u(x)$ was calculated from the standard deviation of 10 replicates of pipette dispense volumes obtained from pipette calibration data.

Table 4. Estimation of the contribution of volume to the uncertainty of measurement in each of the major steps of the Abbott Murex HCV EIA procedure.

Description (abbreviation)	Value (x)	Pipette used	Standard Deviation of 10 replicates $u(x)$	Relative Standard Uncertainty (RSD)
	μL	μL		$\frac{u(x)}{x}$
Reconstitution of Conjugate	20,000	NA		
Reconstitution of Substrate	35,000	NA		
Sample diluent Vol_{dil}	180	200	0.085	0.000472222
Sample/control $Vol_{s\&c}$	20	20	0.085	0.00425
Conjugate Vol_{conj}	100	100	0.083	0.00083
Substrate Vol_{sub}	100	100	0.083	0.00083
Stop solution Vol_{stop}	50	50	0.083	0.00166

The total RSD of volume (RSD_v) was calculated by combining the RSD of each component of volume.

	$u(Vol_{dil})$	$u(Vol_{s\&c})$	$u(Vol_{conj})$	$u(Vol_{sub})$	$u(Vol_{stop})$	Total
RSD	0.000472222	0.00425	0.00083	0.00083	0.00166	
RSD^2	2.22994E-07	1.80625E-05	6.889E-07	6.889E-07	2.76E-06	
$\sum RSD^2$						2.24189E-05
$\sqrt{\sum RSD^2}$						0.004734859

Combined Relative Standard Uncertainty of volume (RSD_v) = 0.004734859

5.4. Reading

5.4.1. Absorbance of test

The absorbance of the test is read twice, once at 450 nm and at a reference reading of 620 nm. The absorbance reading at 620 nm is subtracted from the 450 nm reading.

Using the results of 10 readings of a single channel of the reader at 450 nm using the calibrated glass filter with a target value (x) of 1.119, the standard deviation $u(x)$ was 0.000446.

$$\text{Relative standard uncertainty} = \left(\frac{u(x)}{x} \right) = \frac{0.000446}{1.119} = 0.00039857$$

Using EURACHEM / CITAC Guide Rule 1 (8.2.6.): For models involving only sum or a difference of quantities e.g. $y = (p + q + r + \dots)$, the combined relative standard uncertainty $u(y)$ is given by:

$$u(y[p, q, r, \dots]) = \sqrt{u(p)^2 + u(q)^2 + u(r)^2 + \dots}$$

As the reading is performed twice, the combined reading relative standard uncertainty or reading is

$$\sqrt{0.00039857^2 + 0.00039857^2} = 0.000563663$$

Combined Relative Standard Uncertainty of read ($RSD_{450 \text{ and } 620}$) = 0.000563663

5.4.2. Spectral absorbance of glass filters

If limits of $\pm a$ are given with a confidence limit (in the form of $\pm a$ at $p\%$) then divide the value a by the appropriate percentage of the Normal distribution for the level of confidence given to calculate the standard deviation (EURACHEM / CITAC Guideline 8.1.3.). The 95% CI is calculated using a value of 1.96σ .

The MultiSkan Ascent Plate reader is calibrated using three glass filters that have known optical values and uncertainty. At 450 nm the 02044/1 filter has an absorbance of 0.5580 ± 0.0029 (95% CI).

The standard uncertainty of the glass filter calibration is $\frac{0.0029}{1.96} = 0.00148$

The relative standard uncertainty of the 02044/1 filter at 450 nm (target value $(x) = 0.6$) is

$$RSD_{\text{filter}} = \frac{u(x)}{x} = \frac{0.00148}{0.6} = 0.002466667$$

5.4.3. Combined standard uncertainty

Combined standard uncertainty of reading is

$$u_{\text{read}} = \sqrt{0.000563663^2 + 0.002466667^2} = 0.002530249$$

Combined Relative Standard Uncertainty of reading (RSD_R) = 0.002530249

5.5 Additional MU Components

The following components contributing to UM have been estimated to identify the extent of their contribution to total uncertainty. These three components are 1) Operator, 2) Reagent batch and 3) Bias between laboratories. In estimating the contribution of these three components, it is understood that

- Each of the three components include variation contributed by Time, Temperature, Volume and Readings
- There is double-counting of Time, Temperature, Volume and Reading

Wherever possible, the variation contributed by Operator, Reagent batch and Bias have been calculated independent to each other by careful selection of data populations. The estimations of the contribution to variation contributed by Operator, Reagent batch and Bias are included in this document to provide an estimate of the measure of their significance to MU.

5.5.1 Operator

Two operators from a single laboratory tested a low positive QC sample in a single batch of reagent. Therefore the major contribution to variation was due to the processes used by the operators. There was noticeable variation between the results reported by these two operators (Table 5), an uncommon finding. Therefore these results represent the greatest possible variation between operators.

Table 5. The results reported by two operators from the same laboratory testing the same low positive QC sample multiple times.

Operator	1	2
Number of results (n_n)	10	11
Mean of S/Co results (x_n)	3.3740	1.5755
Standard deviation of results (s_n)	0.3295	0.1828
RSD $\left[\frac{u(x)}{x} \right]$	0.098	0.116

From a series of estimates of standard deviation on replicate results of similar samples, an estimate of overall method standard deviation may be calculates using the formula [4],

$$S_r^2 = \frac{\sum (n-1)S^2}{[\sum n] - p}$$

where

S_r	=	Overall standard deviation for the method
n	=	number of replicates
p	=	number of samples
$[\sum n] - p$	=	degrees of freedom

Therefore the pooled RSD of the operator can be estimated using the formula [4]

$$RSD_{operat} = \sqrt{\frac{(n_1 - 1) \left(\frac{s_1}{x_1} \right)^2 + (n_2 - 1) \left(\frac{s_2}{x_2} \right)^2}{n_1 + n_2 - 2}} = 0.1077$$

Relative Standard Uncertainty of Operator (RSD_{oper}) = 0.1077

5.5. Reagent batches

A single operator tested a low positive QC sample in three batches of reagent in the same laboratory. Therefore the major contribution to variation was due to the change in reactivity due to the batch of reagent. The results are presented in Table 6.

Table 6. The results of a low positive QC sample tested multiple times in two reagent batches in the same laboratory and tested by the same operator.

Reagent batch	1	2	3
Number of results (n)	10	8	9
Mean of S/Co results (\bar{x})	3.3740	3.1913	3.6000
Standard deviation of results $u(\bar{x})$	0.3295	0.8060	0.3766
RSD $\left[\frac{u(\bar{x})}{\bar{x}} \right]$	0.0977	0.2526	0.1046

Using the formula $S_r^2 = \frac{\sum (n-1)S^2}{[\sum n] - p}$

$$RSD_{operat} = \sqrt{\frac{(n_1 - 1) \left(\frac{s_1}{x_1} \right)^2 + (n_2 - 1) \left(\frac{s_2}{x_2} \right)^2 + (n_3 - 1) \left(\frac{s_3}{x_3} \right)^2}{n_1 + n_2 + n_3 - 3}} = 0.3809$$

Relative Standard Uncertainty of reagent ($RSD_{reagent}$) = 0.3809

5.6. Bias between laboratories

Bias is traditionally defined as the difference between the **observed result** and the **expected (or true) result**. In order to eliminate the units of measurement and use the quotient rule for combining uncertainty, a measure of bias can be expressed as a ratio as in recovery experiments.

$$bias = \frac{\text{Observed result}}{\text{Expected result}} = \frac{X_{obs}}{X_{exp}} \quad \text{where } X_{exp} \text{ is the agreed true value}$$

The uncertainty of bias (u_{bias}) can be calculated using the formula:

$$RSD_{bias} = \frac{u_{bias}}{bias} = \sqrt{\left(\frac{u_{obs}}{X_{obs}} \right)^2 + \left(\frac{u_{exp}}{X_{exp}} \right)^2} \quad \text{or} \quad \sqrt{RSD_{obs}^2 + RSD_{exp}^2}$$

Where u_{obs} is the standard deviation of a laboratory's results and X_{obs} is the mean of a laboratory's results

and

$$RSD_{\text{exp}} = \frac{S_{\text{exp}}}{X_{\text{exp}}}$$

The RSD_{exp} can be calculated using the results of all laboratories testing the same QC sample and applying a weighting inversely proportional to the respective variance of the mean of each laboratory's results [5]

$$W_{\text{lab1}} = \frac{N_{\text{lab1}}}{S_{\text{lab1}}^2} \quad W_{\text{lab2}} = \frac{N_{\text{lab2}}}{S_{\text{lab2}}^2} \quad W_{\text{labn}} = \frac{N_{\text{labn}}}{S_{\text{labn}}^2}$$

where W_{labn} is the weighting inversely according to the respective variance of the means
 N_{labn} is the number of results in the population used to calculate the mean labn and
 S_{labn}^2 is the variance of the population used to calculate the mean of labn

The expected mean result can therefore be calculated using the formula

$$\text{Mean}_{\text{exp}}(X_{\text{exp}}) = \frac{W_{\text{lab1}}X_{\text{lab1}} + W_{\text{lab2}}X_{\text{lab2}} + \dots\dots\dots W_{\text{labn}}X_{\text{labn}}}{W_{\text{lab1}} + W_{\text{lab2}} + \dots\dots\dots + W_{\text{labn}}}$$

where X_{labn} is the mean of the results contributed to the population by labn

Using the same weighting principle [5], the standard deviation of all results in the total population (S_{exp} or u_{exp}) is calculated by

$$S_{\text{exp}} = u_{\text{exp}} = \sqrt{\frac{1}{W_{\text{lab1}} + W_{\text{lab2}} + \dots\dots\dots + W_{\text{labn}}}}$$

The relative standard uncertainty of the expected results (RSD_{exp}) is calculated by:

$$RSD_{\text{exp}} = \frac{S_{\text{exp}}}{X_{\text{exp}}} \quad \text{from above.}$$

6.0. Standard Uncertainty of all Variables

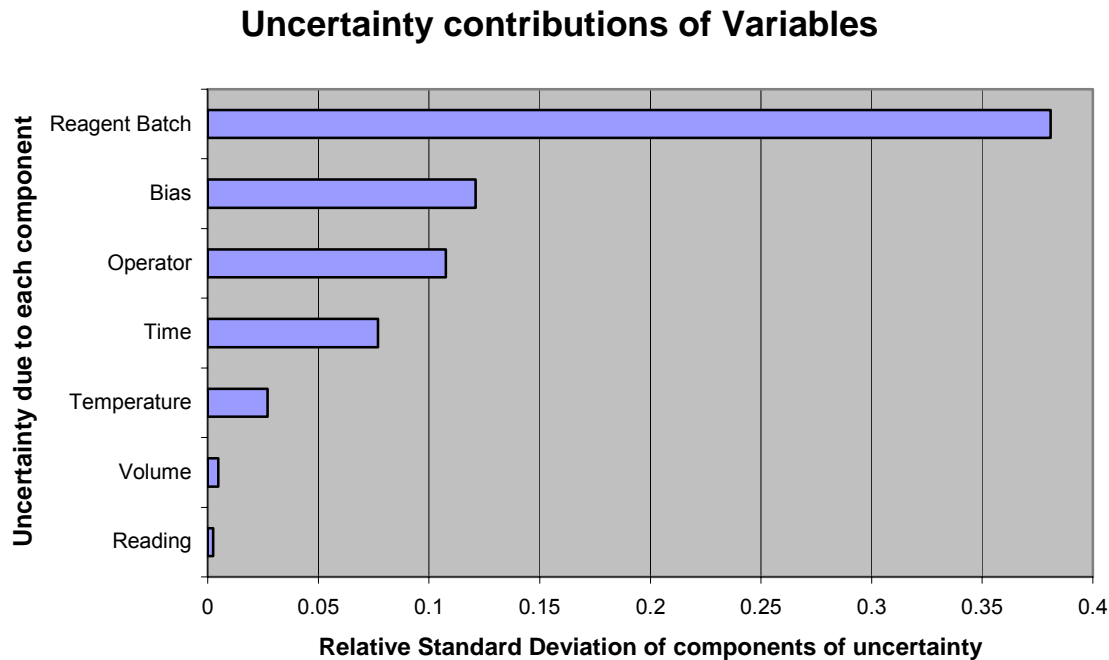
The individual combined relative standard uncertainties of each of the components estimated above are:

- $RSD_{\text{time}} = 0.076923077$
- $RSD_{\text{temp}} = 0.027027027$
- $RSD_{\text{vol}} = 0.004734859$
- $RSD_{\text{read}} = 0.002530249$
- $RSD_{\text{oper}} = 0.1077$
- $RSD_{\text{reagent}} = 0.3809$
- $RSD_{\text{bias}} = 0.121$ (as calculated for Lab 14 – not shown)

7.0 Uncertainty contribution of all variables

The contribution of variation for each component has been calculated and is graphed below (Figure 2). Although all measures have been taken to minimise double counting, components such as operator and batch variation will include elements of time, volume, reading and temperature. These calculations provide an estimation of the contribution to the total variation by each component.

Figure 2. Graphical display of the contribution of each source of variation identified in an Abbott Murex HCV EIA.



As these calculations may include double counting of temperature, time, volume and reading, addition of these components to derive a total variation will result in a MU estimate greater than reality. However, these estimations provide a means of identifying which elements contribute significantly to the total variation. It can be determined that reagent batch, laboratory to laboratory variation and operator variation contribute significantly to the total variation in this test system whereas the reading, volume and temperature contribute less to the total variation. Therefore estimation of total MU requires these components to be considered.

8.0 Combination of relative standard uncertainties

Combining the relative standard uncertainties using the formula

$$u(y(p, q, \dots)) = \sqrt{RSD_p^2 + RSD_q^2 + \dots}$$

$$RSD_{combined} = \sqrt{RSD_{time}^2 + RSD_{temp}^2 + RSD_{vol}^2 + RSD_{read}^2 + RSD_{oper}^2 + RSD_{reagent}^2 + RSD_{bias}^2} = 0.421902$$

To determine the expanded uncertainty ($U_{RESULT[95\% CI]}$) the U_{obs} is multiplied by a coverage factor of 2

$$\text{Expanded Uncertainty } U_{RESULT[95\% CI]} = 0.421902 \times 2 = 0.84.$$

9.0 Comparison of calculated Uncertainty of Measurement with real QC data

A low-level quality control sample was tested by 18 different laboratories 2167 times (Number of tests per laboratory range = 3 -1190). When the results of all laboratories were combined, the standard deviation of all 2167 results was 0.74; mean 2.67. The range of the standard deviation (precision) of individual laboratories was 0.33 to 2.0

Using the 2167 test results of a single sample tested by 18 laboratories using multiple batches of reagent, and weighting the calculation to take into account the number of tests conducted by individual laboratories and the laboratory's precision, an expanded uncertainty can be calculated. (See document titled "Calculating Uncertainty of Measurement using Precision and Bias) [6].

10.0 Results

Table 7. QC sample, MULTI:SER:08, was tested in the Murex anti-HCV (version 4.0) in laboratories designated by number. The instrument, number of observations, mean and variations around the mean over the period 01/01/1999 to 20/09/2004 are shown.

Lab	n	Mean	SD	%CV	RSD_{prec}	Weighting (W)	W x Mean	Expanded Uncertainty
1	31	3.32	0.04	12.05	0.12	194	643.25	1.07
2	98	3.51	0.97	27.64	0.28	104	365.59	2.14
3	16	3.52	0.36	10.23	0.10	123	434.57	1.17
4	3	2.30	0.40	17.39	0.17	19	43.13	0.90
5	44	2.42	0.52	21.49	0.21	163	393.79	1.07
6	64	3.18	0.42	13.21	0.13	363	1153.74	1.01
7	27	3.32	0.62	18.67	0.19	70	233.19	1.44
8	48	2.88	0.33	11.46	0.11	441	1269.42	0.72
9	32	2.67	0.55	20.60	0.21	106	282.45	1.12
10	3	1.77	0.37	20.90	0.21	22	38.79	1.21
11	14	3.13	0.66	21.09	0.21	32	100.60	1.46
12	36	3.28	2.00	60.98	0.61	9	29.52	4.09
13	54	2.71	0.44	16.24	0.16	279	755.89	0.89
14	284	3.25	0.71	21.85	0.22	563	1830.99	1.56
15	1190	2.39	0.50	20.92	0.21	4760	11376.40	1.03
16	99	2.30	0.76	33.04	0.33	171	394.22	1.56
17	54	3.11	0.59	18.97	0.19	155	482.45	1.29
18	70	2.69	0.38	14.13	0.14	485	1304.02	0.77
Total	2167	2.67	0.74	27.72		8059	21131.98	

All components of variation in a testing system are included when the results of a QC sample are used to determine MU and include both the imprecision and the bias contributed by reading, temperature, time, volume, reagent batches, operator and laboratory bias. Precision is a measure of the ability of a laboratory to reproduce the same result each time the same sample is tested. Bias is a measure of the ability of a laboratory to get a "true result" when it tests a sample.

By using a combination of Precision and Bias generated from the QC results, an expanded uncertainty can be calculated for each individual laboratory, which ranges from 0.72 to 4.09.

11.0 Conclusion

The RSD_{Prec} of the laboratories (Table 7) are a measure of their Precision. The values range from 0.10 to 0.61. Using Precision alone as a measure of MU ($RSD_{Prec} \times 2$ - Expanded Uncertainty), the values of MU for the 18 laboratories range from 0.2 – 1.22 (average = 0.4). This value does not take into account any systemic variation that was experienced by the laboratories. For example, if the ambient temperature is 18 °C in one laboratory testing an assay and 29 °C in another laboratory using the same assay, a bias may be detected in the results reported by the two laboratories, even though the ambient temperatures are within the manufacturer's specified limits.

Combining Precision and Bias as a measure of MU, the expanded uncertainty ranges from 0.72 – 4.0 (average 1.4). Excluding two outliers (4.0 and 2.14) the average expanded uncertainty was 1.14, which is close to the calculated expanded uncertainty derived from the GUM analysis above (0.84).

Performing a full GUM analysis on each individual assay would be a time consuming process. Often, appropriate data are not available and many assumptions are required. For example, using GUM, a mathematical estimation of the effects on a test system contributed by time and temperature can be estimated. However, there is no modelling available to determine that the estimated effect actually occurs. A rise of 2 °C during incubation cannot be directly related to a specific change in the reactivity of a sample. A more direct method of estimating MU was required.

A well-chosen QC sample, tested in the same manner as a patient sample, will undergo the same variation as experienced by the patient samples. The QC sample must closely resemble real samples and have reactivity close to the medical decision-making level. The results of such a QC samples can be used to estimate MU in serological assay as long as both Precision and Bias are taken into account. With Precision alone, the MU will be underestimated. Using Precision and Bias will provide laboratories a more accessible method to estimate MU compared with the GUM approach. A more detailed description of an approach to calculate MU using Precision and Bias is detailed in another document [6].

12.0 Acknowledgements

The Serology Uncertainty of Measurement Working Party is comprised of

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