**Supplemental Material 1. Basic metrological terminologies, definitions and symbols.**

|  |  |
| --- | --- |
| Metrological terminology (symbol) | Definition |
| Measurand | Quantity intended to be measured. |
| Measurement uncertainty | Non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used. |
| Uncertainty budget | Statement of a measurement uncertainty, of the components of that measurement uncertainty, and of their calculation and combination |
| Standard measurement uncertainty | Measurement uncertainty expressed as a standard deviation. |
| Relative standard measurement uncertainty | Standard measurement uncertainty divided by the absolute value of the measured quantity value different from zero |
| Combined standard measurement uncertainty | Standard measurement uncertainty that is obtained using the individual standard measurement uncertainties associated with the input quantities in a measurement model. |
| Coverage factor | Number larger than one by which a combined standard measurement uncertainty is multiplied to obtain an expanded measurement uncertainty. |
| Coverage interval | Interval containing the set of true quantity values of a measurand with a stated probability, based on the information available |
| Expanded measurement uncertainty | Product of a combined standard measurement uncertainty and a factor larger than the number one |

**Supplemental Material 2. Estimation of** **uncertainty for imprecision**

In general, internal quality control (IQC) materials are routinely used to ensure the laboratory measurement procedures and assumed to behave like patient samples. If the IQC data are collected during a sufficiently long period time to reflect typical source affecting many routine changes of conditions, the variability of IQC data (imprecision) can be used as an uncertainty budget of intermediate reproducibility condition. The relative uncertainty of imprecision for QC lot (, equal to coefficient of variation) is obtained as following equation:

where is mean of measurement in QC lot and is standard deviation in QC lot . If there are multiple QC changes over a sufficient period of time, the pooled long-term relative uncertainty of imprecision () is calculated as follow:

where is number of QC lots for calculation of pooled uncertainty and is number of QC runs in QC lot .

**Reference**

1. CLSI. Expression of Measurement Uncertainty in Laboratory Medicine; Approved Guideline. CLSI document EP29-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2012

**Supplemental Material 3. Relative uncertainties of imprecision for each analyte**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | | Fibrinogen, mg/dL | | Antithrombin, % | | aPTT, Sec | | PT, Sec | | Protein C, % | | Protein S, % | |
| Measurement instrument | | ACL TOP 750 CTS | | ACL TOP 750 CTS | | ACL TOP 750 CTS | | ACL TOP 750 CTS | | STA Compact | | STA Compact | |
| IQC level | | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 |
| IQC lot 1 dates | | 01/07/2018  ~31/12/2018 | | 01/07/2018  ~31/12/2018 | | 01/07/2018  ~31/12/2018 | | 01/07/2018  ~31/12/2018 | | 01/07/2018  ~02/08/2018 | | 01/07/2018  ~02/08/2018 | |
|  | Number of IQC | 165 | 165 | 92 | 92 | 552 | 552 | 552 | 552 | 5 | 5 | 5 | 5 |
|  | Mean of IQC | 275.3 | 221.8 | 99.4 | 24.0 | 29.0 | 41.9 | 12.1 | 22.3 | 98.6 | 39.6 | 77 | 33.2 |
|  | Standard deviation of IQC | 12.1 | 13.8 | 4.9 | 1.2 | 1.3 | 1.8 | 0.3 | 1.0 | 3.0 | 1.5 | 7.6 | 2.9 |
| IQC lot 2 dates | |  |  |  |  |  |  |  |  | 03/08/2018  ~31/12/2018 | | 03/08/2018  ~31/12/2018 | |
|  | Number of IQC |  |  |  |  |  |  |  |  | 19 | 19 | 19 | 19 |
|  | Mean of IQC |  |  |  |  |  |  |  |  | 96.2 | 39.3 | 73.5 | 29.1 |
|  | Standard deviation of IQC |  |  |  |  |  |  |  |  | 3.3 | 1.6 | 4.5 | 1.2 |
| Relative uncertainty of precision | | 4.4% | 6.2% | 4.9% | 5.0% | 4.4% | 4.2% | 2.4% | 4.6% | 3.4% | 4.0% | 7.0% | 5.4% |

IQC, internal quality control; aPTT, activated partial thromboplastin time; PT, prothrombin times

**Supplemental Material 3. (continued)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | | Factor V, % | | | | Factor VIII, % | | | | Factor X, % | | | |
| Measurement instrument | | ACL TOP 750 CTS | | STA Compact | | ACL TOP 750 CTS | | STA Compact | | ACL TOP 750 CTS | | STA Compact | |
| IQC level | | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 |
| IQC Lot 1 Dates | | 01/07/2018  ~31/12/2018 | | 01/07/2018  ~02/08/2018 | | 01/07/2018  ~31/12/2018 | | 01/07/2018  ~02/08/2018 | | 01/07/2018  ~31/12/2018 | | 01/07/2018  ~02/08/2018 | |
|  | Number of IQC | 19 | 19 | 3 | 3 | 19 | 19 | 3 | 3 | 19 | 19 | 3 | 3 |
|  | Mean of IQC | 97.6 | 31.0 | 86.7 | 32.0 | 90.3 | 30.5 | 98.0 | 41.3 | 92.1 | 29.8 | 86.7 | 41.0 |
|  | Standard deviation of IQC | 2.5 | 1.0 | 2.5 | 2.0 | 3.1 | 1.4 | 3.5 | 1.2 | 1.3 | 0.6 | 0.6 | 1.0 |
| IQC Lot 2 Dates | |  |  | 03/08/2018  ~31/12/2018 | |  |  | 03/08/2018  ~31/12/2018 | |  | | 03/08/2018  ~31/12/2018 | |
|  | Number of IQC |  |  | 16 | 16 |  |  | 16 | 16 |  |  | 16 | 16 |
|  | Mean of IQC |  |  | 81.6 | 33.9 |  |  | 105.2 | 44.4 |  |  | 83.7 | 36.6 |
|  | Standard deviation of IQC |  |  | 4.4 | 2.0 |  |  | 5.5 | 1.6 |  |  | 2.8 | 1.2 |
| Relative uncertainty of precision | | 2.6% | 3.2% | 5.1% | 6.1% | 3.4% | 4.6% | 5.0% | 3.5% | 1.4% | 2.0% | 3.1% | 3.1% |

IQC, internal quality control

**Supplemental Material 4. Assessment of absolute bias for uncertainty calculation**

When available, natural matrix certified reference materials (CRMs) are used for bias evaluations of laboratory procedures. To assess the bias and the measurement uncertainty in bias estimation, CRMs are repeatedly measured for several runs. Table 1 lists the measurement values (), where indexes the runs and indexes the replicates.

**Table 1.** Measured values of CRM

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Measurement runs | Replicate 1 | Replicate 2 | … | Replicate |
| 1 |  |  | … |  |
| 2 |  |  | … |  |
| 3 |  |  | … |  |
| 4 |  |  | … |  |
| … | … | … | … | . |
|  |  |  | … |  |

From one-way analysis of variance, sum of square due to within-run () and sum of square due to between-run () are obtained as following:

where is within-run mean square, is between-run mean square and is number of replicate for each run. Then, standard uncertainty of CRM measurement () is calculated as follow:

The bias () between the mean of CRM measurements () and the certified value of CRM () is

and the combined standard uncertainty of bias () is

where is standard uncertainty of CRM. If the observed bias () is not covered by the expanded uncertainty interval (),

the bias cannot be asserted to be zero and the bias correction should be implemented for the measurement system.

**Reference**

1. CLSI. Expression of Measurement Uncertainty in Laboratory Medicine; Approved Guideline. CLSI document EP29-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

**Supplemental Material 5. Bias assessment using results of interlaboratory comparison study**

The biases from interlaboratory comparison studies can be used to check the evaluated uncertainty in the same way as a reference material. In order to include the spread of biases over interlaboratory comparison rounds, a laboratory should participate at least 6 times within a reasonable interval. Biases from interlaboratory comparison study can be both positive and negative, and all bias values can be used for estimating the uncertainty component as follow:

where is root mean square of bias, is bias from the -th interlaboratory comparison, and is number of interlaboratory comparisons. The, the standard uncertainty of interlaboratory comparison () is

where is between laboratory standard deviation and is number of participated laboratories. Then, the combined uncertainty of bias () is

**Reference**

1. EUROLAB. EUROLAB Technical Report No. 1/2007. EUROLAB Technical Report. 2007.
2. Magnusson B, Näykki T, Hovind H, Krysell M. Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories. Oslo, Norway: Nordic Innovation; 2012.

**Supplemental Material 6. Bottom-up approaches for international normalized ratio (INR)**

The definition of INR is “patient’s prothrombin time (PT) test result expressed as a ratio to a normal population (MNPT) which has been standardized (or normalized) for the potency of the thromboplastin used in the assay”. There are two calibration models adopted by the WHO; local test system ISI calibration and “Direct” INR determination. Both models based on mathematical equations for calculating local INR values, and the law of propagation of uncertainty enables the quantitation of the combined uncertainty on the results. If measurand is calculated from following equation:

where is value of variable, then the combined measurement uncertainty of measurand () is obtained according to the law of propagation of uncertainty as followings:

where is standard uncertainty of each variable and is sensitivity coefficient of ().

For local ISI calibration model, the PT values of the certified plasma are determined using the local thromboplastin and reference thromboplastin. The ISI value calculated from local ISI calibration () is obtained by plotting the logarithm of PTs with orthogonal regression analysis as followings:

where is PT using reference thromboplastin, is PT using local thromboplastin, and are the intercept and slope of orthogonal regression analysis, and is assigned value of ISI for the reference thromboplastin reagent. Then, local INR result determined with local ISI calibration model () is

It is important to note that orthogonal regression line should ideally pass through normal and abnormal PT observations because the regression line describes the relationship between PT results from normal and abnormal plasmas. If there is marked deviation, the local ISI calibration would not be meaningful. In this case, INR of local system is estimated by following equations:

which is referred by ‘Tomenson’s model’. is a scale parameter, and are PT value and MNPT of local system, and is assigned value of ISI for reference thromboplastin. The scale parameter is estimated as

where and are the mean logarithms of normal PTs determined with local thromboplastin and reference thromboplastin, and and are the intercept and slope of the abnormal-only orthogonal regression line. Generally, , the mean logarithms of normal PTs determined with the local system is equal to the natural logarithm of , and the following equation is obtained:

According to the law of uncertainty propagation, the combined measurement uncertainty can be calculated from following equations:

where and are the standard error of the intercept and the slope , is standard error of determined with reference thromboplastin, is pooled long-term imprecision of local PT system, and is standard uncertainty of reference thromboplastin. Then, the expanded uncertainty using a coverage factor of 2 is given by

(, about 95% coverage probability)

In the direct INR determination method, INR is determined from a PT/INR calibration line using certified plasmas without employing ISI and MNPT of local PT measurement system. The reference INR values determined with reference thromboplastin is calculated by

where and are PT result and MNPT determined with reference thromboplastin, and is certified value of ISI for the reference thromboplastin. The linear relationship exists between the natural logarithms of and PT of local system (), and is determined using the following orthogonal regression formula:

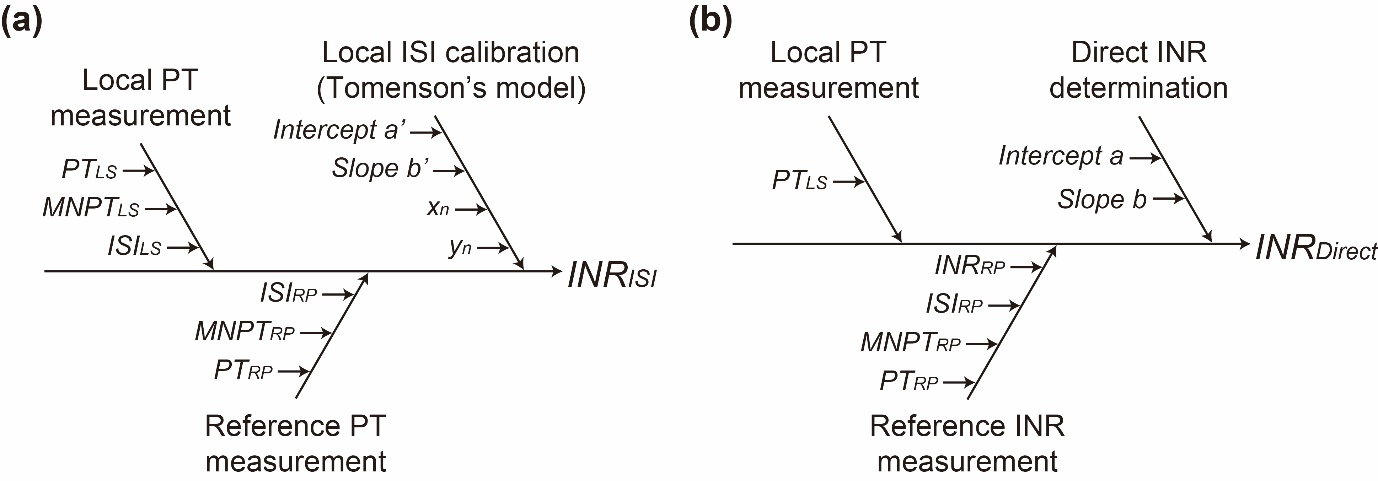
where and are the intercept and slope of orthogonal regression analysis. The intercept and slope with each measurement uncertainties (standard errors of intercept and slope)are calculated with orthogonal linear regression formulas. Then, the combined uncertainty of is quantified as followings:

As same as local ISI calibration method, the expanded uncertainty is calculated as follows:

(, about 95% coverage probability)

The relevant uncertainty sources for local ISI calibration method and direct INR determination method are summarized in the cause and effect diagram at Figure 1.

**Figure 1.** Cause and effect diagrams for local ISI calibration method (a) and direct INR determination method (b).



**Reference**

1. ISO. ISO 17593:2007 Clinical laboratory testing and in vitro medical devices - Requirements for in vitro monitoring systems for self-testing of oral anticoagulant therapy. Geneva, Switzerland: ISO; 2007.
2. Chantarangkul V, Tripodi A, van den Besselaar A. Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy with vitamin K antagonists. WHO Technical report series. 2013;979:273-305.
3. CLSI. Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline. CLSI document H54-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
4. JCGM. Evaluation of measurement data – Guide to the expression of uncertainty in measurement. Paris, France: JCGM; 2008.
5. Tomenson J. A statistician’s independent evaluation. Thromboplastin calibration and oral anticoagulant control: Springer; 1984;87-108.

**Supplemental Material 7.** **Estimation of measurement uncertainty estimated with top-down approach using interlaboratory comparison results.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Analyte | | | INR | aPTT, Sec | Fibrinogen, mg/dL | Antithrombin, % | Protein C, % | Protein S, % |
| Measurement instrument | | | ACL TOP 750 CTS | ACL TOP 750 CTS | ACL TOP 750 CTS | ACL TOP 750 CTS | STA Compact | STA Compact |
| Interlaboratory comparison material level 1 | | | | | | | | |
|  | Bias estimation using interlaboratory comparison results | | | | | | | |
|  |  | Peer group grand mean | 1.06 | 27.9 | 306.6 | 113.2 | 108.6 | 86.7 |
|  |  | Average uncertainty of EQA results | 0.3% | 0.6% | 0.8% | 0.8% | 0.9% | 1.8% |
|  |  | Root mean square of bias | 2.0% | 5.0% | 4.1% | 0.8% | 1.1% | 6.5% |
|  |  | Relative uncertainty of bias value | 2.0% | 5.1% | 4.2% | 1.1% | 1.4% | 6.7% |
|  | Relative uncertainty of precision | | 2.4% | 4.4% | 4.4% | 4.9% | 3.4% | 7.0% |
|  | Relative expanded uncertainty a | | 6.3% | 13.5% | 12.2% | 10.1% | 7.3% | 19.4% |
| Interlaboratory comparison material level 2 | | | | | | | | |
|  | Bias estimation using interlaboratory comparison results | | | | | | | |
|  |  | Peer group grand mean | 2.80 | 58.0 | 120.1 | 56.7 | 36.6 | 35.1 |
|  |  | Average uncertainty of EQA results | 0.4% | 0.8% | 1.1% | 1.0% | 0.7% | 2.1% |
|  |  | Root mean square of bias | 5.7% | 6.2% | 5.7% | 3.1% | 1.8% | 5.2% |
|  |  | Relative uncertainty of bias value | 5.7% | 6.3% | 5.8% | 3.3% | 1.9% | 5.6% |
|  | Relative uncertainty of precision | | 4.6% | 4.2% | 6.2% | 5.0% | 4.0% | 5.4% |
|  | Relative expanded uncertainty a | | 14.6% | 15.1% | 17.0% | 12.0% | 8.8% | 15.6% |
| Interlaboratory comparison material level 3 | | | | | | | | |
|  | Bias estimation using interlaboratory comparison results | | | | | | | |
|  |  | Peer group grand mean | 4.47 | 79.5 | 83.8 | 34.8 | 23.3 | 24.5 |
|  |  | Average uncertainty of EQA results | 0.5% | 0.5% | 1.5% | 2.4% | 1.1% | 1.5% |
|  |  | Root mean square of bias | 5.6% | 1.6% | 7.6% | 4.2% | 3.3% | 6.3% |
|  |  | Relative uncertainty of bias value | 5.6% | 1.7% | 7.7% | 4.8% | 3.5% | 6.5% |
|  | Relative uncertainty of precision | | 4.6% | 4.2% | 6.2% | 5.0% | 4.0% | 5.4% |
|  | Relative expanded uncertainty a | | 14.5% | 9.0% | 21.3% | 13.9% | 10.5% | 16.9% |

aThe relative expanded uncertainty was obtained by multiplying the relative combined uncertainty with a coverage factor (, about 95% coverage probability).

aPTT, activated partial thromboplastin time; INR, international normalised ratio; EQA, proficiency testing/external quality assessment

**Supplemental Material 8. Log-log plot of prothrombin times and international normalized ratio (INR) for local international sensitivity index determination (a) and direct INR determination (b).**

