1. Purpose
1.1 To provide the procedure for defining and evaluating analytical performance within NSW Health Pathology laboratories offering chemical pathology services.

2. Background
2.1 This procedure defines best practice in:
   2.1.1 The establishment of analytical performance goals
   2.1.2 The calculation and presentation of measurement uncertainty and
   2.1.3 The assessment of lot-to-lot variation.

3. Scope
3.1 This procedure is mandatory for managers and scientific staff providing chemical pathology services within NSW Health Pathology.
3.2 Each laboratory must establish and demonstrate evidence of ongoing assessment of analytical performance.

4. Definitions
4.1 CLSI: Clinical Laboratory Standards Institute.
4.2 CV: Coefficient of Variation is the standard deviation divided by the mean. It is often expressed as a percentage.

5. Procedure
5.1 Analytical Performance Goals
   5.1.1 Evaluating analytical performance provides an understanding of how good the analytical procedure needs to be in order for the test to be clinically meaningful and useful.
   5.1.2 By defining performance requirements a priori, it is then possible to decide whether the performance of an analytical process is satisfactory.

5.2 Total Error
   5.2.1 One of three different models must be used to define total error.
       a) Model 1: Based on the effect of analytical performance on clinical outcomes:
          i. Direct outcome studies: Investigating the impact of analytical performance of the test on clinical outcomes or
          ii. Indirect outcome studies: Investigating the impact of analytical performance of the test on clinical classifications or decisions and thereby on the probability of patient outcomes
       b) Model 2: Based on components of biological variation of the measurand, and
       c) Model 3: Based on state-of-the-art.
5.2.2 The model used depends on the information available for the assay being evaluated.

5.2.3 The Royal College of Pathologists of Australia Quality Assurance Program \(^3\) allowable limits of performance \(^8\) will be used for establishing total error where this information is available.

5.2.4 Where these have not been defined, the performance goals may be calculated based on biological variation using the equations outlined below.

5.2.5 When using biological variation data, total error is given by \(^4\).

\[
TE = [k \times 0.5 \times CV_w] + [0.25 \times \sqrt{CV_w^2 + CV_g^2}]
\]

\(k = 1.65\) for 95% confidence (unidirectional)

\(CV_w = \) Within individual biological variation

\(CV_g = \) Between individual biological variation

This error budget includes components for imprecision and bias such that:

\(CV_a < 0.5 \times CV_w\), and

\[
\text{Bias} < 0.25 \times \sqrt{CV_w^2 + CV_g^2}
\]

where \(CV_a\) is the analytical variation.

For therapeutic drugs, assumptions about steady state fluctuations between some minimum and some maximum can be used as surrogate for biological variation. In this way, precision requirements may be defined as:

\[CV_a < 0.25 \times \frac{2^{T/t} - 1}{2^{T/t} + 1} \times 100\%
\]

where \(CV_a\) is the analytical variation, \(T\) is the dosing interval, and \(t\) is the half-life.

5.2.6 Where information is not available from either of these approaches, then performance may be compared to the current state-of-the-art.

5.2.7 A spreadsheet for use in setting total error accompanies this procedure.

5.3 Measurement Uncertainty

5.3.1 All types of measurement have some inaccuracy due to bias and imprecision. The dispersion of results obtained from repeated measurements (imprecision) can be described approximately by a normal probability (Gaussian) distribution, with some 95% of the results falling within \(\pm 2\) standard deviations (SD) of the mean value.

5.3.2 The current concept of measurement uncertainty (MU) assumes that significant bias has been corrected or eliminated \(^5\). Some knowledge of the result variability expected from a given measurement procedure is required if results are to be meaningfully compared with other results of the same kind or with decision and legal limits.

5.3.3 ISO/IEC Guide 98-3:2008 Guide to the Expression of Uncertainty in Measurement describes the approach for estimating and expressing measurement uncertainty. In the simplest case, where the uncertainties are independent of one another, these are calculated as the square root of the sum of the squares of the uncertainties (root mean square).
5.3.4 The requirements for the estimation of measurement uncertainty with which NSW Health Pathology laboratories must comply are outlined by NPAAC.

5.3.5 Clinical biochemistry measurement methods employ quality control (QC) materials to estimate and monitor whole procedure imprecision. QC data can be used to estimate the contribution of random effects to the measurement uncertainty of the whole procedure, with the assumption that the measurand behaves identically in both patient samples and quality control material.

5.3.6 If a procedure has been adjusted for bias, then the uncertainty associated with the correction may need to be combined with the measure of imprecision to estimate MU for the procedure.

5.3.7 All laboratories must estimate measurement uncertainty for all procedures where relevant and where possible.

5.3.8 MU only needs to be estimated for the laboratory, not each individual instrument. A NSW Health Pathology Network should be able to demonstrate equivalent performance on identical platforms, in which case the same MU estimation may be used for each individual instrument.

5.3.9 Where a patient sample may be directed to more than one instrument within a laboratory, MU must consider uncertainty arising from between instrument variation.

5.3.10 MU should be estimated for all calculated tests such as adjusted calcium, eGFR, and creatinine clearance. The contributing uncertainties can be assumed to be independent so the combined uncertainty will be the square root of the sum of the squares of the component uncertainties. For example, where anion gap is calculated as:

\[ AG = [Na] + [K] - [Cl] - [HCO3] \]

Then \( U_{AG} = (U_{Na})^2 + (U_K)^2 + (U_{Cl})^2 + (U_{HCO3})^2)^{1/2} \)

5.3.11 Records for MU for quantitative assays must include the information shown in Table 1 - Minimum MU Information Needed for Quantitative Assays.

<table>
<thead>
<tr>
<th>Measurand</th>
<th>This includes the quantity intended to be measured; system or specimen type; and kind-of-quantity. For example, concentration of creatinine in serum or plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Units</td>
<td>The unit of measure. Irrespective of whether mass or molar units are used, concentrations should be expressed with the denominator as the base unit, for example, ug/L not mg/mL</td>
</tr>
<tr>
<td>Method</td>
<td>The analytical principle used</td>
</tr>
<tr>
<td>Measurement Procedure</td>
<td>Including the instrument used</td>
</tr>
<tr>
<td>Test Limitations and Significant Interferences</td>
<td>Note significant interference from haemolysis, lipaemia or icterus when relevant</td>
</tr>
<tr>
<td>Calibrator Traceability</td>
<td>Generally provided by the supplier</td>
</tr>
<tr>
<td>Calibrator Uncertainty</td>
<td>Generally provided by the supplier</td>
</tr>
<tr>
<td>Bias</td>
<td>Only if not assumed to be negligible based on the manufacturers method of calibration</td>
</tr>
<tr>
<td>Imprecision</td>
<td>This should include the concentration or activity at which measurements were assessed in addition to SD and CV</td>
</tr>
<tr>
<td>Estimated MU</td>
<td>Including imprecision, calibrator uncertainty and between instrument uncertainty where relevant</td>
</tr>
<tr>
<td>Analytical Goal</td>
<td>The analytical goal and comparison to the goal to assess performance</td>
</tr>
<tr>
<td>Fit for Purpose</td>
<td>Statement of whether the assay is fit for purpose and any necessary actions</td>
</tr>
</tbody>
</table>
5.3.12 Records for MU for quantitative assays must include reference change values estimates.

a) The reference change value (RCV) is the minimum difference between serial results that must be exceeded before those results can be considered to be significantly different from one another with a prescribed level of confidence. Although not required the RCV may be calculated as shown below.

The RCV for a significant change at the 95% level of confidence is:

\[ RCV = \sqrt{2} \times 1.96 \times \sqrt{CV_a^2 + CV_i^2}, \]

Where, \( CV_a \) is the analytical imprecision and \( CV_i \) is within subject biological variation.

b) The number of significant figures to be displayed on pathology reports is also dependent on the RCV. For example, the apparent difference between reported figures of 6.2 and 6.1 may obscure a true difference as little as 0.01 (6.15 – 6.14) or as much as 0.19 (6.24 – 6.05). If the RCV is \( >= 0.19 \) then no additional information is given by the second decimal place.

5.3.13 MU estimations must be performed at a maximum interval of not greater than six-months.

5.3.14 A template for evaluation of measurement accompanies this procedure.

5.4 Reagent Lot-to-Lot Variation

5.4.1 The goal of both reagent manufacturers and clinical laboratories is to provide accurate patient results. However, an unexpected change in performance with a new reagent lot may occur for QC and/or patient samples.

5.4.2 Reagent manufacturers use a number of procedures to validate the performance of a new reagent lot during the manufacturing process. Nevertheless, the laboratory must verify that the new reagent lot, as received, meets the laboratory’s clinical performance needs.

5.4.3 Possible causes of a change in performance with a new reagent lot include:

(a) Changes in reagent component materials
(b) Instability of a component in a reagent
(c) Reagents compromised in transportation or storage, and
(d) Incorrect calibration of the new reagent lot.

5.4.4 Verifying that these potential changes have not occurred is important to assure the quality of laboratory results and is therefore a time-limited procedure.

5.4.5 Between-reagent lot variation can affect results for QC materials and/or patient samples.

5.4.6 For some measurements, reagent lot variation is observed in results for QC materials even though there has not been a significant change in patient sample results.

5.4.7 However, a systematic change in QC results may not be immediately apparent, but may become recognised only after a number of QC results have been accumulated over a period of time while using a new reagent lot. This variation for QC results is often ascribed to “matrix effects” which suggests that the QC material is not commutable with fresh patient samples.

5.4.8 The manufacturing process for QC materials has a significant impact on the matrix of samples and the reagent manufacturer’s first concerns must be accuracy and consistency with patient sample results.
5.4.9 However, it cannot be assumed that the absence of a lot-to-lot difference in the results obtained from QC samples is proof that no such difference exists with patient samples.

5.4.10 This situation may occur because the magnitude of matrix-related differences for the QC material is different for each reagent lot. The differences may offset each other to appear as if no change has occurred when a change exists for patient samples. Such a difference in patient results will not be detected if only QC materials are used during crossover testing when reagent lots are changed.

5.4.11 In addition, QC material supplied with the reagents may be "optimised" to perform correctly with each new reagent lot. In that circumstance, performance of the new reagent lot with the supplied QC material may not reflect performance with patient samples.

5.4.12 Therefore, new reagent lots be assessed, and reagent lot-to-lot evaluations be performed using patient samples for all reagent lot changes.

5.4.13 Assessment should occur before, or concurrent with, initial use of any new reagent lot for patient testing.

5.4.14 **Process for Assessing Lot-to-Lot Variation**

   a) The evaluating laboratory must have access to adequate numbers of appropriate clinical samples for evaluation, sufficient instrument and technologist time for the evaluation, and sufficient stock of previously verified reagents so that the verification procedure is not emergent.

   b) Assessing lot-to-lot variation does not need to be repeated across different laboratories for a single lot number. Therefore, to minimise time and cost, it would be prudent for laboratories within a single network, and using the same equipment, to distribute the workload for assessing lot-to-lot variations equitably.

   c) Laboratory management must define and document the acceptable limits for patient results when changing a lot of a reagent for all of the measurement procedures that are being evaluated.

   d) The procedure requires running a small comparison study of 10 to 20 patient samples and ensure that (i) the differences are not statistically different from zero, (ii) the difference are all less than an allowable limit of performance, and (iii) the average difference is less than 1/3 the within-subject biological variation.

   e) A template for evaluation of lot-to-lot variation accompanies this procedure.

6. **Roles and Responsibilities**

6.1 Managers are responsible for ensuring staff are aware of, and trained in the use of, this procedure. Managers are also responsible for ensuring that records are retained according to NPAAC requirements.

6.2 Scientific staff must use the lot-to-lot evaluation process referred to in this procedure when introducing new reagent lots into the laboratory.

6.3 Scientific staff are responsible for documenting the results of the evaluation.
7. Legal and Policy Framework


4. Fraser, CG, Biological Variation: From Principles to Practice, AACC Press, Washington, 2001


8. Review

This procedure will be reviewed by 30/06/2017.

9. Risk

<table>
<thead>
<tr>
<th>Risk Statement</th>
<th>If test results do not meet minimum analytical performance criteria due to ineffective monitoring and processes, the consequences could result in harm to patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Category</td>
<td>Clinical Care and Patient Safety</td>
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</table>
10. Further Information
For further information, please contact:

<table>
<thead>
<tr>
<th>Policy Contact Officer</th>
<th>Position: Chair, Chemical Pathology Clinical Stream</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Name: Fernando San Gil</td>
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<tr>
<td></td>
<td>Telephone: 02 4222 5405</td>
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<td></td>
<td>Email: <a href="mailto:Fernando.SanGil@health.nsw.gov.au">Fernando.SanGil@health.nsw.gov.au</a></td>
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11. Version History
The approval and amendment history for this document must be listed in the following table.

<table>
<thead>
<tr>
<th>Version No</th>
<th>Effective Date</th>
<th>Approved By</th>
<th>Approval Date</th>
<th>Risk Rating</th>
<th>Sections Modified</th>
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<tbody>
<tr>
<td>1.0</td>
<td>26/09/16</td>
<td>NSWHP ELT</td>
<td>11/05/16</td>
<td>Medium</td>
<td>New Procedure</td>
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<tr>
<td>1.1</td>
<td>14/11/16</td>
<td>NSWHP CE</td>
<td>14/11/16</td>
<td>Medium</td>
<td>5.3.12 - equation changed to CVa squared added to the CVi squared; NSWHP SD_007, NSWHP SD_008 and NSWHP SD_009 - RCPA QAP information added to FT3, FT4, Thyroglobulin, PTH. In NSWHP SD_008 protected fields in the “Data Entry Sheet” have also been unlocked.</td>
</tr>
</tbody>
</table>

12. Attachments
12.1 Template for Evaluation of Total Error NSWHP SD_007
12.2 Template for Measurement Uncertainty (MU) NSWHP SD_008
12.3 Template for Evaluation of Lot-to-Lot Variation NSWHP SD_009