

Procedure

Analytical Performance in Chemical Pathology

NSWHP_PR_018

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 The establishment of analytical performance specifications
 - 2.2 The calculation and presentation of measurement uncertainty, and
 - 2.3 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.

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5.1 Analytical Performance Specifications

- 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 ² 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.

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- 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 Australasia Quality Assurance Program ³ allowable limits of performance ³ 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, desirable 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:

$$CV_a < 0.5 \times CV_w, \text{ and}$$

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

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:

$$CV_a < 0.25 \left(\frac{2^{T/t} - 1}{2^{T/t} + 1} \right) 100\%$$

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 ± 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).

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- 5.3.4 The requirements for the estimation of measurement uncertainty with which NSW Health Pathology laboratories must comply are outlined by NPAAC ⁵.
- 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 ⁶.
- 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 laboratory 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:
$$AG = [Na] + [K] - [Cl] - [HCO_3]$$

Then
$$U_{AG} = ((U_{Na})^2 + (U_K)^2 + (U_{Cl})^2 + (U_{HCO_3})^2)^{1/2}$$
- 5.3.11 Records for MU for quantitative assays must include the information shown in the following table:

Table 1 - Minimum MU Information Needed for Quantitative Assays

Measurand	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
Units	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
Method	The analytical principle used
Measurement Procedure	Including the instrument used
Test Limitations and Significant Interferences	Note significant interference from haemolysis, lipaemia or icterus when relevant
Calibrator Traceability	Generally provided by the supplier
Calibrator Uncertainty	Generally provided by the supplier
Bias	Only if not assumed to be negligible based on the manufacturers method of calibration
Imprecision	This should include the concentration or activity at which measurements were assessed in addition to SD and CV
Standard Uncertainty	Including imprecision, calibrator uncertainty and between instrument uncertainty where relevant
Expanded Uncertainty	Standard Uncertainty x Coverage Factor. In most instances the coverage factor will be set to 1.96 to provide a 95% confidence interval.
Analytical Performance Specification	The analytical performance specification (APS) and comparison to the specification to assess performance. This will typically be expressed as a sigma metric or capability index where $\sigma = APS/Standard\ Uncertainty$
Fit for Purpose	Statement of whether the assay is fit for purpose and any necessary actions

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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:

$$RCV = \sqrt{2} * 1.96 * \sqrt{CV_a^2 * CV_i^2},$$

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 ⁷.

5.3.13 MU estimations must be performed at a maximum interval of not greater than 12months.

5.3.14 A [template](#) for evaluation of measurement accompanies this procedure.

5.4 Reagent or Calibrator Lot-to-Lot Variation (LTLV)

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 or calibrator 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 or calibrator lot during the manufacturing process. Nevertheless, the laboratory must verify that the new reagent or calibrator lot, as received, meets the laboratory's clinical performance needs.

5.4.3 Possible causes of a change in performance with a new reagent or calibrator lot include ⁸.

- (a) Changes in component materials
- (b) Instability of a component in a reagent or calibrator
- (c) Reagents or calibrator compromised in transportation or storage, and
- (d) Incorrect calibration of the new or current 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 or calibrator lot variation can affect results for QC materials and/or patient samples.

5.4.6 For some measurements, reagent or calibrator 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 or calibrator 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.

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- 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 or calibrator lots must be assessed, LTLV evaluations be performed using patient samples for all reagent or calibrator 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 performing LTLV evaluation**
- a) as a minimum a LTLV evaluation should be performed when:
 - The assay has a clear decision limits that defines a condition, or is used as treatment target, or the assay is used for long-term monitoring. For these assays long term stability of results is essential.
 - The assay is known to exhibit significant lot-to-lot variability_e.g. Lipase for Roche platforms.
 - The assay uses QC that is not commutable with patient samples and hence may mask a shift e.g. analytes from non-human source.
 - b) 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.
 - c) 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.
 - d) 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.
 - e) The procedure requires running a small comparison study of a range of 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.
 - f) A [template](#) for evaluating limited patient comparison data accompanies this procedure and is suitable for the assessment of lot-to-lot variation.

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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 ⁹.
- 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

1. Kenny D, Fraser CG, Hyltoft Petersen P, Kallner A, Strategies to set global analytical quality specifications in laboratory medicine – Consensus agreement, Scan J Clin Lab Invest, 1999;59:585 <http://www.tandfonline.com/toc/iclb20/59/7>
2. Sverre S, Fraser CG, Horvath AR, Jansen R, Jones G, Oosterhuis W, Hyltoft Petersen P, Schimmel H, Sikaris K, and Panteghini M, Defining analytical performance specifications, Consensus statement from the 1st strategic conference of the European Federation of Clinical Chemistry and Laboratory Medicine, Clin Chem Lab Med, 2015, DOI: 10.1515/cclm-2015-0067 <http://www.ncbi.nlm.nih.gov/pubmed/25719329>
3. Royal College of Pathologists of Australasia Quality Assurance Program <http://www.rcpaqap.com.au/docs/2014/chempath/ALP.pdf>, Accessed 14th April 2016.
4. Fraser, CG, Biological Variation: From Principles to Practice, AACC Press, Washington, 2001
5. National Pathology Accreditation Advisory Council, Requirements for the Estimation of Measurement Uncertainty, Australian Government Department of Health and Ageing, Canberra, 2007, <http://www.health.gov.au/internet/main/publishing.nsf/Content/health-npaac-emu.htm>
6. White, GH, Basics of estimating measurement uncertainty, Clinical Biochemist Reviews, 2008;29 (Suppl i):S53-S60, <http://www.ncbi.nlm.nih.gov/pubmed/18852859>
7. Hawkins RCW, Johnson RN, The significance of significant figures, Clinical Chemistry, 1990;36(5):824, <http://www.clinchem.org/content/36/5/824.1.full.pdf+html>
8. Clinical and Laboratory Standards Institute, User Evaluation of Between-Reagent Lot Variation; Approved Guideline EP26-A, CLSI, Pennsylvania, 2013, <http://shop.clsi.org/method-evaluation-documents/EP26.html>.
9. National Pathology Accreditation Advisory Council, Requirements for the Retention of Laboratory Records and Diagnostic Material, Australian Government Department of Health and Ageing, Canberra, 2013, <http://www.health.gov.au/internet/main/publishing.nsf/Content/health-npaac-docs-RetLabRecDI.htm>, Accessed 14th April 2016.

8. Review

This procedure will be reviewed by 30/06/2020.

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9. Risk

Risk Statement	If test results do not meet minimum analytical performance criteria due to ineffective monitoring and processes, the consequences could result in harm to patients.
Risk Category	Clinical Care and Patient Safety

10. Further Information

For further information, please contact:

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11. Version History

The approval and amendment history for this document must be listed in the following table.

Version No	Effective Date	Approved By	Approval Date	Risk Rating	Sections Modified
1.0	26/09/16	NSWHP ELT	11/05/16	Medium	New Procedure
2.0	14/11/16	NSWHP CE	14/11/16	Medium	5.3.12 - equation changed to CVa squared <u>added to</u> 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.
3.0	04/12/18	Clinical Governance, Quality and Risk Committee	04/12/18	Medium	New policy template; Change of contact; 5.3.11 minor name change; 5.4.14 key factor that render the assessment of LTLV essential.; Spreadsheets reduced from 3 to 2 for useability.
4.0	25/06/19	Executive Director, Strategy & Transformation	25/06/19	Medium	Fix broken hyperlink at 12.1.

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12. Attachments

- 12.1 [Template for Evaluation of Measurement Uncertainty \(MU\) NSWHP_SD_008](#)
- 12.2 [Template for Evaluation of Limited Patient Comparison Data NSWHP_SD_009](#)
(Use for the Assessment of Lot-to-Lot Variation)