The Royal College of Pathologists
Pathology: the science behind the cure

ISO 15189:2012 – An approach to the assessment of uncertainty of measurement for cellular pathology laboratories

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Background

This document has been produced at the request of the Specialty Advisory Committee (SAC) for Cellular Pathology, to provide laboratories with an approach to the assessment of the uncertainty of measurement, an area of quality assurance with which they may be unfamiliar. The document has been discussed with the United Kingdom Accreditation Service (UKAS) assessment team so that we move towards a shared understanding of the most appropriate ways to meet this aspect of the ISO 15189 standard.

The document was discussed and agreed at the April 2015 meeting of the SAC for Cellular Pathology and at the meeting of the MedLab Technical Advisory Committee of UKAS (April 2015).

Although written specifically for cellular pathology, other specialties may also find the information useful.

1 Introduction

1.1 ISO standards

As laboratories move from CPA accreditation to UKAS accreditation under ISO 15189:2012, staff will be required to assess critically various aspects of their work. This paper highlights one area, that of clause 5.5.1.4 in the ISO standard, which concerns the uncertainty of measured quantity values.

"The laboratory shall determine measurement uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patients' samples. The laboratory shall define the performance requirements for the measurement uncertainty of each measurement procedure and regularly review estimates of measurement uncertainty."

A second ISO standard, ISO 17025:2005, is a normative reference for ISO 15189:2012 and provides more detailed information on the approach to this issue, which suggests a pragmatic way through the potential problems for cellular pathology.

ISO 17025:2005, clause 5.4.6.2 states:

"Testing laboratories shall have and shall apply procedures for estimating uncertainty of measurement. In certain cases the nature of the test method may preclude rigorous, metrologically and statistically valid, calculation of uncertainty of measurement. In these cases the laboratory shall at least attempt to identify all the components of uncertainty and make a reasonable estimation, and shall ensure that the form of reporting of the result does not give a wrong impression of the uncertainty. Reasonable estimation shall be based on knowledge of the performance of the method and on the measurement scope and shall make use of, for example, previous experience and validation data."

1.2 Cellular pathology

For cases where measurements are made on sequential samples from a patient, there is a requirement to assess whether a difference in results is due to the uncertainty of measurement or real variation in the parameter being assessed. This is rarely the case in cellular pathology, where each specimen is unique and cannot be considered to be homogeneous in the way that, for example, a blood sample is homogeneous – measurements on ten aliquots from one blood sample are likely to give very similar values, while measurement on ten tissue sections are likely to differ.

For tissue samples, the measurement is often being compared to the evidence on which staging schemes and treatment protocols have been devised. It is appropriate **to consider** the potential uncertainty of measurement of the value obtained in the laboratory and how that may impact on prognostic or predictive thresholds; the practical issues involved in this consideration are outlined later.

In simple terms, where a measurement provided by the laboratory potentially has a direct clinical impact, the laboratory should be able to demonstrate that they have **considered** the uncertainty of that measurement. The way in which a laboratory does this will vary according to the specific context. However, there will be occasions within medical laboratories where, due to the heterogenous nature of the sample, uncertainty and the associated uncertainty budgets (type A and type B) cannot be determined (see below).

1.3 Definitions

Measurement: A measurement is a property of something and gives a number to that property. Measurement usually involves a measuring instrument (ruler, balance, thermometer, etc).

Counting is not normally viewed as a measurement.

A test (in this context, the delivery of a diagnostic opinion on a tissue sample) is not normally viewed as a measurement as the result is pass/fail or yes/no (based on evidence and experience). However, measurements may be involved in determining the outcome of a test.

Uncertainty of measurement expresses (i.e. attempts to quantify) the doubt that inevitably exists when any measurement is made. In most circumstances, this relies on statistical data from repeated measurements that allow one to state the degree of confidence that a measured value lies within a certain range (e.g. a length is 50 ± 1 cm with 95% confidence).

Error is the difference between a measured value and its 'true value'; where possible one tries to correct errors by appropriate calibration. This is the same as the precision of a measurement (i.e. is this the right answer). Precision is assessed under a different clause in ISO 15189:2012 and in general requires reference to an external calibration standard/control material.

2 General principles

2.1 Uncertainty evaluation

ISO 17025:2005 is the technical standard that underpins ISO 15189, and many assessors will be working to this standard. UKAS document M3003 provides some guidance and clarification on ISO 17025. Laboratories should therefore seek to explain their process of 'consideration of uncertainty' in terms which technical assessors will understand. It is likely that unless you can express the uncertainty of measurement in statistical terms (type A evaluation), appropriate 'consideration' will lead you to a conclusion that there are too many variables in the process to express the uncertainty in a meaningful way. Each laboratory is expected to consider the matter for relevant measurements. Examples of such considerations are provided in Section 3 and Appendix.

Type A uncertainty evaluation is carried out using the statistical analysis of a series of observations and is useful for assessing random variations in a series of measurements. This is most appropriate for numerical data based on a group of patients or where results from multiple samples from one patient can be compared (for example, using the arithmetic mean, standard deviation, confidence intervals). This is rarely the situation in cellular pathology.

Type B uncertainty evaluation is used for systematic factors that may affect measurements. For tissue samples, these may include fixation time and temperature, processing schedules, sectioning and staining techniques and the process of measurement.

Such uncertainty budgets will need to be interpreted in the context of what is clinically relevant in a particular type of case.

2.2 Which measurements are and are not relevant?

For the purposes of this ISO clause, the clinical opinion is not being measured. Therefore, any uncertainties associated with the pathologist's opinion that the diagnosis is, for example, carcinoma, are not relevant. Diagnostic uncertainties can be expressed in the text of a report (including comment on such variables as the amount of lesional material, and artefacts associated with the biopsy procedure or processing) where appropriate. The quality and competence of a pathologist is considered in other RCPath documents and assessed under a different ISO 15189:2012 clause.

Where weights and measurements are part of an overall description and do not impart prognostic or predictive value, assessment of the uncertainty of measurement is inappropriate.

The size of a carcinoma, when used to assess its stage, is a critical measurement, therefore it is appropriate to consider the uncertainty of its measurement. See Section 3 and Appendix.

Mitotic counts and grading are numerical data based on subjective assessments that do not normally require detailed measurement of uncertainty (for a worked example see below). Laboratories should consider whether or not normative referencing, i.e. comparison of grading patterns between colleagues within or between centres, might be an alternative way of providing reassurance on the quality of local assessments; such normative referencing falls outside the scope of clause 5.5.1.4, but could still be regarded as good practice.

2.3 Communication of uncertainty with users

A laboratory only needs to make its estimates of uncertainty available on request. If a user is requesting this information in the first place, one might assume that they have a reasonable understanding of what uncertainty is and why it will occur. There will be an uncertainty to all measured values and in many circumstances quoting estimates of uncertainty on every report might introduce undesirable ambiguity. Whether a user has a good understanding or not, the key point is that the laboratory should consider whether or not they can quantify the uncertainty and relate this to any defined performance requirements.

Laboratories should maintain a central record of how they have considered uncertainty of measurement, e.g. on a document management system. This is a resource that can be used to respond to requests for information.

2.4 Source of uncertainty

In general, the following factors may affect a measurement:

- the measuring instrument – maintenance and calibration
- the object being measured – is the measured property stable or not (fixation)?
- the measurement process may be inherently difficult (tumour size in three dimensions)
- the environment – temperature, air pressure, humidity
- sampling issues – where and when to measure
- the operator's skill and judgement (training and competence).

Many factors may affect the measurement obtained during a pathological assessment. Some uncertainties are an inevitable consequence of the removal of tissue from a patient and its handling in the laboratory. Laboratories should avoid an inappropriate degree of precision in reporting measurements.

2.4.1 Estimates of size, for example of tumours, will vary according to whether they are made by imaging *in vivo*, macroscopically or microscopically. Tumours tend to reduce in size on excision as blood flow ceases, fixation and processing will tend to reduce size, and section cutting and staining will often increase apparent size (floating out on waterbaths).

2.4.2 Distance to resection margins is subject to error where tissues retract after excision (e.g. colon, muscle, mucosal resections) or where tissues are easily distorted (e.g. fatty breast tissue). Margins involving more solid tissues (e.g. fibrous tissue, liver) are more predictable. The 'final' histological distance to a margin is, in some circumstances, of less relevance than the nature of the tissue and the surgical quality of the margin. An opinion as to whether or not a lesion is completely excised is normally expected for a surgical resection.

2.4.3 Pragmatically, the College's cancer datasets have used macroscopic dimensions, confirmed or adjusted on the basis of microscopy. This is a useful principle, not least because this is the basis on which prognostic data in clinico-pathological studies tend to be based. It should be recognised that most prognostic markers studies have not considered the uncertainty of measurement when threshold values have been defined.

2.4.4 pT staging and FIGO staging are usually provisional results and may be moderated in multidisciplinary discussion where all the circumstances of the cases are known.

2.4.5 Where evidence-based national or international methods are available, these should be referred to in local guidance (e.g. KI67 assessment of proliferation and HER-2 assessment in breast carcinomas.

Dowsett M, Nielsen TO, A'Hern R, Bartlett J *et al*. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. *J Natl Cancer Inst* 2011;103(22):1656–1664.

Wolff AC, Hammond MEH, Hicks DG, Dowsett M *et al*. Recommendations for human epidermal growth factor receptor 2 testing breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice update. *J Clin Oncol* 2013;31(31):3997–4013.

3 Site-specific and context-specific aspects

3.1 Weights of tissue/organs in surgical pathology

- These generally fall into the 'good description' category as they are not usually of prognostic or predictive relevance.

- Weights of parathyroids should be measured in milligrams. Weighing to ±5 mg is likely to be sufficient, even for normal glands, but balances that only weigh to 0.01 g are arguably inadequate for this purpose. Balances need to be calibrated regularly (and not just serviced). This may incur a cost for the calibration certificate.

3.2 Weights and measurements of organs and bodies at autopsy

- These generally fall into the 'good description' category as they are not usually of diagnostic importance.

- The possible exception is the weight of the heart and weight or thickness of the ventricular walls, where this is used to assess hypertrophy. However, as these specimens are unique and meticulous dissection to remove adipose tissue is rarely necessary, the true uncertainty of measurement is not possible to define.

3.3 Measurements of tissue/tumour size and distance to margins

- Measurements of tumours are generally used to provide staging and prognostic information, and as such are usually critical measurements.

- The procedure to be followed to determine tumour measurements should be defined in departmental standard operating procedures (SOPs) to ensure consistency (intra- and inter-observer variation should be minimised and can be audited).

- Macroscopically, the measurements are usually made with rulers. Laboratories should consider whether or not the ruler needs to be calibrated against a standard. Consideration should also be given to reducing observer variation by performing repeated (x3) measurements on single specimens. If consideration indicates that these problems in assessment are not likely to be of significance, then a pragmatic view suggests that it is not necessary to expend effort in determining how accurately calibrated is a ruler (see Appendix).

- Where measurements are made microscopically, evidence of calibration of the equipment used to make the measurement (e.g. Vernier scale or eyepiece graticule) **sufficient to meet clinical requirements** should be available. Again, consistency studies should be considered.

3.4 Numerical counts of immunocytochemically positive cells (receptors, proliferation)

- These numerical counts do not specifically require an estimate of uncertainty as they are not measurements as defined in ISO17025. Nevertheless, sufficient nuclei/cells should be assessed to give a reliable estimate. Consider national guidance where available (see 2.4.5).

- The method to be used when performing these counts should be defined in departmental SOPs to ensure consistency. Factors such as variation in fixation and antigen retrieval methods can significantly influence these tests and could be monitored through internal laboratory quality control processes. Validation and verification of the performance of automated platforms for immunocytochemistry will form part of the overall assessment of uncertainty.

- Whether these numerical assessments are made by the pathologist's brain or by image analysis software, the laboratory should consider how many cells should be counted to provide an accurate representation of the whole lesion? Does this vary between tumour types? Should we consider hotspots or overall mean values? What is the intra- and inter-observer variation? It is likely that if a Type B uncertainty evaluation is performed that the unknown effects of different sources of uncertainty will make a precise estimate of the uncertainty of measurement impossible.

3.5 Numerical counts of gene and chromosome copy number on slide based technologies, e.g. ISH for HER-2

- National guidance exists for some of these assessments (see 2.4.5) and should be reflected in departmental SOPs. These numerical counts do not specifically require an estimate of uncertainty as they are not measurements as defined in ISO17025. Nevertheless, sufficient nuclei should be assessed to give a reliable estimate.

3.6 Numerical estimates of gene copy number based on PCR technology, e.g. OSNA

- The manufacturers should provide validation data on their machines and the user should have verified these data. Although specimens are unique, as the tissue are homogenised, it is possible to take several aliquots of the homogenate from one sample and derive a mathematical expression of uncertainty. Routine daily calibration and quality assessment against external standards should provide sufficient data for this purpose.

3.7 Cytology-specific areas

- Some technologies require the measurement of relative light units for the testing of human papilloma virus status. Assessment of the error of this measurement should form part of the validation and verification process for the equipment.

- Measurements are performed within the BD Source Bioscience scanner to determine the risk of abnormality when used in 'no further review' mode. These data is not available to the laboratory and thus assessment of the error of this measurement must be referred to the company.

3.8 Cancer datasets and tissue pathways

- Where measurements are of critical importance for diagnosis, prognosis or prediction of therapeutic response, dataset authors should consider providing guidance on the assessment of uncertainty of measurement in the datasets and tissue pathways.

4 Summary

To facilitate compliance with ISO 15189:2012, laboratories should do the following.

1. Consider which reported measured values are clinically critical (rather than descriptive) and hence require consideration of the uncertainty of measurement to be available to users and UKAS assessors on request.

2. In cellular pathology, there will rarely be the opportunity to obtain mathematical data that are sufficiently robust to describe the uncertainty of measurement as intended by ISO17025:2005. A more descriptive evaluation (type B) of uncertainty is likely to be appropriate.

3. The lack of tissue homogeneity in most cellular pathology material makes it impossible to determine the 'true value' of a measurement. It is therefore not possible to estimate the uncertainty of the measurement and a pragmatic approach is appropriate based on published literature and clinical experience.

4. Although the College is providing general guidance through this document, each laboratory should consider the relevant aspects for themselves. Even though a formal expression of the uncertainties may not be possible, laboratories should seek to reduce the uncertainties through:

    - consideration of which are the best methods to achieve clinically reliable measurements and ensure these are defined in departmental SOPs

    - working to ensure that the measurement procedures are consistent between pathologists

- ensuring that equipment used is calibrated regularly to a traceable standard. Traceable calibrations can be sourced in the following ways:
  - calibration laboratories that have been assessed and accredited by UKAS (or another signatory to the multi-lateral agreement). In these cases the calibration certificate will carry the UKAS (or international equivalent) symbol
  - by the medical laboratory performing in house calibrations, which are then assessed by UKAS (to ISO/IEC 17025) as part of their assessment to ISO 15189
- assessing regularly that the use of the equipment achieves the desired objectives
- maintaining process records of this activity in a secure location for assessment under ISO15189:2012.

Appendix Worked examples for illustrative purposes

A1 Weight of fresh parathyroid glands

The principle being that individual glands weighing more than 50 mg are likely to be abnormal with the excess glandular tissue secreting parathormone.

Source of uncertainty	Estimate of effect
Type of balance and calibration	Risk assessment required of equipment related variation
Dissection of attached fat not appropriate	Fat around the gland may account for variable proportion of tissue weighed (often up to 50%)
Variability of composition of parathyroids, including variable component of adipocytes	Some abnormal glands contain non-secretory tissue (fat, fibrous tissue)
Temperature	Unknown
Drying of tissue between theatre and laboratory	Unknown

Conclusion: Precise estimate of uncertainty of weighing is not possible.
Pragmatically, work to SOP and provide best estimate of weight to nearest 10 mg.

A2 Macroscopic assessment of size of neoplasm

Source of uncertainty	Estimate of effect
Reduction in size of tissues after removal from body due to loss of blood volume	Unknown
Reduction in size of tissue when fixed in formalin (not relevant if tissues are assessed unfixed)	10–20% decrease depending on tissue
Possible inaccurate calibration of ruler	Unknown but probably <1% inaccuracy which is minimal in the overall scheme of measurement.
Technical difficulties in assessing three dimensional sizes of some neoplasms, including distortion of tissues by handling	Unknown (10–20%, possibly).
Lack of homogeneity of the tissue means that there will be random effects due to the precise plane of slicing the tissue.	Unknown (10–20%, possibly)
Observer variation in technique and experience	Unknown (10–20%, possibly)
Temperature	Probably minimal

Conclusion: Precise estimate of uncertainty of measurement is not possible, mainly due to lack of homogeneity and no known ‘true’ value.
Each specimen is unique and, pragmatically, work to SOP and provide best estimate possible.

A3 Microscopic assessment of size of neoplasm/margins

Source of uncertainty	Estimate of effect
Changes in size of tissues during fixation, processing and section cutting	Unknown; factors work in different ways to decrease or increase size of measurand
Possible inaccurate calibration of eyepiece graticule or Vernier scale (microscopic measurement device)	Unknown but probably small
Observer variation in technique and experience	Unknown
Lack of homogeneity of the tissue means that there will be random effects due to the precise plane of sectioning of the tissue.	Unknown (10–20%, possibly)

Conclusion: Precise estimate of uncertainty of measurement is not possible, mainly due to lack of homogeneity and no known 'true' value.
Each specimen is unique and, pragmatically, work to SOP and provide best estimate possible.

A4 Microscopic assessment of mitotic counts

Mitotic rate is usually expressed as a mitotic index of x mitoses per microscope field or unit area. One therefore has to consider both the uncertainty in identifying mitoses and the uncertainty in the measurement of the field of view of the microscope or the area of tissue assessed. In practice, precise counts are rarely appropriate and we are concerned with major differences, e.g. between 1–2 mitoses and >10 mitoses in 2 mm^2.

Source of uncertainty	Estimate of effect
Changes in size of tissues during fixation, processing and section cutting	Unknown; factors work in different ways to decrease or increase size of the tissue which, in turn, will influence the area assessed.
Possible inaccurate measurement of field of view; this may be specified by the manufacturer of the microscope (needs verification), measured in-house or for digital platforms may be linked to the viewing software.	Unknown but probably small in relation to other variables
Lack of homogeneity of the tissue means that there will be random effects due to the precise plane of sectioning of the tissue.	Unknown (10–20%, possibly)
Observer variation in technique and experience; different observers vary in their assessments of mitotic index, and selection of areas of tissue for assessment.	Unknown

Conclusion: Precise estimate of uncertainty of measurement is not possible, mainly due to lack of homogeneity and no known 'true' value.
Each specimen is unique and, pragmatically, work to SOP and provide best estimate possible.

A5 Microscopic assessment of Ki67 proliferation index

The proliferation rate of a tissue may be expressed as a Ki67 index of x positive nuclei per microscope field or unit area. One therefore has to consider both the uncertainty in identifying positive nuclei and the uncertainty in the measurement of the field of view of the microscope or the area of tissue assessed.

Source of uncertainty	**Estimate of effect**
Changes in size of tissues during fixation, processing and section cutting	Unknown; factors work in different ways to decrease or increase size of the tissue which, in turn, will influence the area assessed.
Possible inaccurate measurement of field of view; this may be specified by the manufacturer of the microscope (needs verification), measured in-house or for digital platforms may be linked to the viewing software.	Unknown but probably small
Lack of homogeneity of the tissue means that there will be random effects due to the precise plane of sectioning of the tissue.	Unknown (10–20%, possibly)
Observer variation in technique and experience; different observers vary in their assessments of Ki67 index, and selection of areas of tissue for assessment.	Unknown

Conclusion: Precise estimate of uncertainty of measurement is not possible, mainly due to lack of homogeneity and no known ‘true’ value.
Each specimen is unique and, pragmatically, work to SOP and provide best estimate possible.