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REVIEW ARTICLE



# Uncertainty of measurement in andrology: UK best practice guideline from the Association of Biomedical Andrologists

D Sanders[a], S Fensome-Rimmer[b] and B Woodward[a]

[a]Association of Biomedical Andrologists, Andrology Department, Heart of England NHS Foundation Trust, Heartlands Hospital, Birmingham, UK; [b]Consulting Quality Scientist

**ABSTRACT**

Uncertainty of measurement has become a paramount factor to consider in pathology. In the UK, consideration of uncertainty of measurement is mandatory for medical laboratories who apply to be accredited against ISO15189:2012 via the United Kingdom Accreditation Service. This guideline intends to help those working within diagnostic andrology to better understand the concept of uncertainty, and how it can be applied to semen analysis and post-vasectomy semen analysis. The various areas where uncertainty may exist are identified, and guidance is provided to minimise this uncertainty. This guidance is produced by the Association of Biomedical Andrologists alongside experts in the field of andrology, in order to aid laboratory scientists in understanding and undertaking important tasks that will improve quality of their service.

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## Introduction

Uncertainty of measurement has become a paramount factor to consider in pathology, as described in various ISO standards, such as ISO17025:2005 [1] and ISO15189:2012 [2]. ISO17025:2005 [1] is the normative reference of ISO15189:2012 [2] and is imperative to understanding uncertainty. The United Kingdom Accreditation Service has also published a helpful document entitled M3003: The Expression of Uncertainty and Confidence in Measurement [3]. Relevant clauses about uncertainty from the two ISO documents are quoted below.

ISO 15189:2012 [2] clause 5.5.1.4 states that:

> 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.

ISO17025:2005 [1] clause 5.4.6.2 states that:

> 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.

Whilst this information is useful, the majority is biased towards chemistry and haematology, rather than andrology. However, by understanding the uncertainty associated with a given value, as measured by andrologists, we are better placed to determine how close the measured value is likely to be to the actual value.

This guideline intends to help those working within andrology to better understand the concept of uncertainty, and how it can be applied to semen analysis (SA) and post-vasectomy semen analysis (PVSA). Potential pitfalls that andrology laboratories may fall into will be identified, and advice provided as to how to address them.

## Discussion

### *Type A and Type B uncertainty*

Uncertainty can be divided into two types: Type A and Type B. Type A uncertainty is evaluated by statistical analysis of data obtained from repeated observations made under the same conditions. Given sufficient measurements, a spread or scatter of results will be observed. Type B uncertainty is an estimation of uncertainty based on data other than by repeated observations. As such, Type B uncertainties are heuristics, or uncertainties that

**CONTACT** B Woodward enquiries@aba.uk.net

do not have a numerical value assigned to them, as described in M3003 [3].

Data in the Type B group can be taken from a number of sources, including previous measurement data, e.g. data from External Quality Assurance (EQA) or Internal Quality Control (IQC), previous experience or knowledge, manufacturer's specifications, calibration certificates and specifically the uncertainty data they provided, uncertainties assigned to reference data. Given the appropriate knowledge and experience to interpret the data provided, evaluation of Type A and B uncertainty can be equally reliable [4].

### *Semen is heterogenous*

Many specimen samples diagnosed by chemistry and haematology laboratories are homogeneous. In contrast, seminal fluid lacks homogeneity and is classed as a heterogenous biological fluid [5]. Another relevant ISO document to consider here is ISO 13528:2015 – Statistical methods for use in proficiency testing by inter-laboratory comparison [6]. From this ISO, clauses 7.1.2, 7.2 and Appendix B.2.3 provide guidance on the process of ascertaining whether a fluid is homogeneous.

Some might consider that uncertainty of measurement cannot be applied to heterogenous fluids. However, consideration of uncertainty can help guide service users in their interpretation of the reported values of a SA or PVSA. For example, knowledge of the dispersal of results around the clinical decision value can help SA interpretation and clinical management.

Doubt may also be cast over the value of uncertainty of measurement if SA is defined from a single test. The SA report summarises a group of individual tests to provide an indication of the fertility potential for an individual. Each parameter is assessed independently of the other parameters. Determining uncertainty of measurement for SA does not devalue the information within the report. Rather, it is only with knowledge of the uncertainty of the reported values, that the information contained with the report can be interpreted in a meaningful manner.

### *Responsibilities*

It is the responsibility of the testing laboratory to identify and to minimise factors affecting the parameters to be tested, and therefore, reduce uncertainty. It is important to provide clear instructions to the patient about sample production. These include the need for hygiene, not using lubricants and collection into a batch controlled specimen container provided by the andrology laboratory. For patients who produce samples 'off-site' (e.g. at home rather than at the hospital), information about transportation conditions and the time to deliver the sample to the laboratory are also crucial. To reduce uncertainty, the laboratory should check with the patient to confirm he has complied with the instructions when delivering the sample.

A simple way to reduce uncertainty regarding the transportation temperature and delivery time is to provide 'on-site' facilities for patients to produce their samples. The World Health Organization laboratory manual for the examination and processing of human semen [7], Section 2.2.1 states: 'The sample should be collected in a private room near the laboratory, in order to limit the exposure of the semen to fluctuations in temperature and to control the time between collection and analysis'.

Regarding the temperature of the motility assessment, the WHO guidelines [7] Section 2.5 note 1 states that motility can be assessed at 'room temperature': 'The procedure may be performed at room temperature or at 37 °C with a heated microscope stage, but should be standardised for each laboratory'. However, 'room temperature' is an ambiguous term, and unless this is constant and the effects on motility at that temperature are understood, the uncertainty that this creates cannot be quantified.

### *Quality control and quality assurance*

The use of EQA samples, both as physical specimens and as digital images, allows the laboratory to assess its performance against defined standards. Uncertainty relating to the EQA provider can be reduced by checking to see if they have been assessed to the ISO standards, such as ISO/IEC 17043:2010 'Conformity assessment – General requirements for proficiency testing' [8]. The consensus or target EQA value is either determined from the trimmed mean of all results received, or from the mean of a sub-population of 'reference' laboratories. These laboratories have been identified as having a consistent historical record of confirmation to specified methodology and having an effective IQC system in place. Therefore, they are likely to demonstrate consistently good performance.

IQC provides an ongoing assessment of individual staff performance and is considered to be more effective than EQA for identifying areas of non-conformity in the assessment process. Uncertainty is reduced if IQC can demonstrate that a semen sample would be assessed to the same level of accuracy by all andrologists in the same laboratory.

### *Uncertainty*

#### *Sources of uncertainty*

Every stage of a SA is subject to uncertainty. It is the responsibility of the laboratory to identify all potential sources of uncertainty. Once identified, the laboratory can then evaluate and attempt to minimise the potential impact of the uncertainty on a reported value.

**Table 1.** A summary of uncertainty factors.

| Stage | Uncertainty factors |
|---|---|
| Pre-examination | Patient preparation (information)<br>Abstinence<br>Method of collection<br>Sample container<br>Transportation<br>Reception |
| Equipment, reagents and consumables | Acceptance<br>Calibration<br>Maintenance<br>Traceability<br>Storage |
| Examination | Verification<br>Validation<br>Documentation |
| Technical | Training<br>Competence |
| Quality control | EQA/Inter-lab comparison<br>IQC |

Many individual stages of the process may indirectly affect a parameter, with multiple stages combining to increase the overall uncertainty of the SA. By summarising individual measurement uncertainty factors, then in theory a measurement uncertainty budget can be produced. This involves describing the measurement procedure and all input variables. However, since andrology often has many Type B uncertainties, where numerical values cannot be given, then an uncertainty statement rather than an uncertainty budget is needed.

Table 1 summarises individual measurement uncertainty factors.

### *Pre-examination*

The laboratory should provide sufficient information to the patient, in a format that is easily understood, so that a sample of an appropriate quality can be obtained. This may require the information to be available in a language other than English. The information should include advice on the abstinence period required, and how the sample should be collected (including the need for personal hygiene and non-use of lubricants) and transported to the laboratory. The sample should be collected into a suitable sample container that has been toxicity-tested using a validated method. Transport to the laboratory should avoid exposure to extremes of temperature.

The WHO guidelines [7] Section 2.2.2 states: 'The specimen container should be kept at ambient temperature, between 20 and 37 °C, to avoid large changes in temperature that may affect the spermatozoa after they are ejaculated into it'.

It is also important that the sample is received by the laboratory within an appropriate period of time, so that it can be processed with a defined time-frame. Specifically, the WHO (2010) guidelines [7] Section 2.3 states: 'Semen analysis should begin … no longer than 1 h after ejaculation, to prevent dehydration or changes in temperature from affecting semen quality'.

If this is not possible, the laboratory needs to consider if on-site production facilities can be provided. On receiving the sample at the laboratory reception, staff should check if the patient has complied with the instructions that the laboratory has provided. It is important to check that the patient has collected a complete semen sample. This is in line with the WHO guidelines [7] Section 2.1, which states: '… losing the first (sperm-rich) portion of the ejaculate has more influence on the results of semen analysis than does losing the last portion'.

It has been reported that some patients may not be entirely honest and may give information that they think the laboratory wants to hear to ensure that the sample is not rejected [9]. The laboratory should therefore, explain to patients why it is important to give factual information, even if it may result in a sample rejection, as incorrect information on sample quality may result in the patient not accessing the correct treatment pathway.

All information provided by the patient should be documented. It is recommended that the patient signs a form to confirm that he has produced the sample and that the information he has provided is correct to the best of their knowledge.

### *Equipment, reagents and consumables*

There is a need to ensure that all items used as part of the analysis are fit for purpose and are used in an appropriate manner. If andrologists are not fully trained to use any item of equipment, this may increase the associated measurement uncertainty. The laboratory should identify all items of equipment that are critical to the reported value. Where an item of equipment has a direct impact on the reported value, it should be calibrated. According to ISO15189:2012 [2], calibration should be traceable through an unbroken chain to a metrological standard. At times, laboratories may use equipment solely for a purpose that does not have a direct impact on the reported value, such as a micropipette that is used to transfer an aliquot of sample to a slide for a motility or morphology assessment. Whilst, it is important that the micropipette should be calibrated to ensure that the desired volume is consistently taken, the laboratory may prefer to use a lower level of calibration. This action may be acceptable, provided that a full risk assessment has been performed to address the issue. However, if the micropipette is used to make a dilution from which the concentration of sperm is determined, a more rigorous calibration is required.

When considering calibration of equipment, it is the responsibility of the laboratory to determine the level of calibration required. It is imperative that the calibration fully covers the range at which the equipment is intended to be used, rather than the full working range of the equipment. For example, if a centrifuge is intended to be used for rotations up to 3000 g, there is negligible value in a calibration at the 6000 g speed.

The service provider for calibration must be accredited to ISO/IEC 17025:2005 [1] for the service that they are providing. It is not sufficient that the equipment that they are using to has been calibrated to ISO 17025:2005 [1]. The laboratory has a responsibility to review the calibration certificates to ensure that the equipment continues to provide the necessary level of accuracy required by the laboratory for the specific assays. This includes determining the frequency for recalibration depending on the frequency of equipment use. Where reagents or consumables are used for the calibration process, the laboratory is required to ensure that these are appropriate for use.

### *Examination process uncertainty*

Choice of methodology is key to managing uncertainty. Where possible, a validated method should be used, accepting that validation does not on its own ensure conformity. The laboratory should then verify that its performance under routine test conditions, with the full team of andrologists, meets the expectations and requirements of the user. If no validated method is available, or the laboratory adapts a validated method to suit its own requirements, then a robust process validation should be undertaken, to ensure that the method achieves the required outcome.

The laboratory should define the biological reference ranges for all of the tests performed, using an accepted source of origin, such as the WHO. Defining the reference range is an important factor when determining the uncertainty of measurement. Without an estimation of uncertainty, it is not possible for physicians to be able to interpret results which are close to the reference range limits. The provision of clear, unambiguous protocols is an important step to ensuring consistency within the laboratory. The protocols should contain all the information required by staff to be able to accurately perform the analysis.

### *Practitioner uncertainty*

Once the laboratory has the appropriate protocols in place, there is a need to ensure that all andrologists are assessed as competent at performing the methods. The laboratory should ensure that there is a robust training programme and ongoing assurance of competence. Training should be supervised by a nominated member of staff who has the necessary skills and expertise. The training process should be documented and progress monitored, allowing the opportunity to review an individual's performance and tailor the training process appropriately. Once training has been completed, the trainees' performance and competence should be assessed by a member of staff with the appropriate authority. Following completion of training, the competence of all staff should be reviewed on a regular basis. The regular assessment of competence ensures that all staff are performing consistently and that the opportunities for deviation are minimised, thereby reducing practitioner uncertainty.

### *Estimating uncertainty*

There is no single approach that will allow the estimation of uncertainty of measurement for every parameter reported. The laboratory should determine the most appropriate method of assessment for each stage of the analysis. Calibration certificates for critical items of equipment should contain information regarding measurement uncertainty. A second source of information can be derived from quality data, both IQA and EQA. This source of information is not without faults, as andrology presently has no controls available containing a known standard. However, by repeated measurements of the same sample, it is possible to determine the most like value of the sample. By increasing the number of replicates, the confidence in the determined value increases.

### *Volume*

Assessment of semen volume can be performed by one of two methods: determination by weighing and volumetric assessment using a serological pipette. The WHO (2010) guidelines [7] Section 2.3.4 recommends weighing, and states: 'The volume is best measured by weighing the sample in the vessel in which it is collected'.

> Measuring volume by aspirating the sample from the specimen container into a pipette or syringe, or decanting it into a measuring cylinder, is not recommended, because not all the sample will be retrieved and the volume will therefore be underestimated.

Determination by weighing relies on the assumption that seminal fluid weighs 1 gram per 1 millilitre. This in itself is an uncertainty, but has been accepted by the WHO guidelines [7] Section 2.3.4 which instructs: 'Calculate the volume from the sample weight, assuming the density of semen to be 1 g/ml' but also adds the comment: 'Semen density varies between 1.043 and 1.102 g/ml'.

Provided the weighing balance is calibrated by an accredited service provider, a calibration certificate should demonstrate the uncertainty associated with the balance. The performance of the balance can then be reviewed on an ongoing basis to ensure that the specifications continue to be met.

Direct measurement of volume has multiple factors that can contribute to uncertainty of measurement. The accuracy of a serological pipette is determined at a specified temperature, normally 20 °C. If the laboratory is going to use the pipette at a temperature that is different from that which the manufacturer states, which is highly probably if the sample is incubated at 37 °C, then the effect of the temperature of the accuracy of measurement should be considered. However, for the time that the sample is in the pipette, this is generally considered to be negligible. Measurement with serological

pipettes requires the operator to be competent in their use, which requires training and regular competence assessment.

Another factor which can impact on the assessment of volume is sample viscosity. It may be considerably more difficult to assess the volume of a high viscous sample, compared to a low viscosity sample which can be easily pipetted.

### *Concentration*

Assessment of concentration involves a combination of factors, all of which can impact on uncertainty. The WHO guidelines [7] recommend using the Improved Neubauer Haemocytometer method, and this requires the preparation of a dilution of the semen sample.

To prepare an accurate dilution requires effective mixing of the sample to attempt to make it as representative of the whole ejaculate as possible. Preparation of the dilution requires the use of two different micropipetters: a positive displacement micropipetter to sample the semen, and an air displacement micropipetter to measure the diluent. Each micropippeter has an uncertainty of measurement associated with it, which will have been determined at calibration and recorded on the calibration certificate.

The diluted sample then requires thorough mixing to ensure complete distribution of the sperm within the diluent. The haemocytometer then needs to be carefully loaded with an appropriately sized tip to minimise any uncertainty related to over- or under-loading. Once loaded, the haemocytometer then needs to be allowed to settle sufficiently in a moist environment, to enable the sperm to be easily counted, but not so long that drying has an effect on the chamber. All of this adds to uncertainty. Whilst, the Improved Neubauer Haemocytometer is considered the 'gold standard' for the assessment of concentration, it is recognised that some laboratories may use an alternative counting chamber to assess sperm concentration. However, it is well documented that other chambers lack the accuracy of the haemocytometer [10–12] and may be subject to either random error or bias. If an alternative counting chamber is used, it should be appropriately validated to ensure the accuracy of its performance and to determine the uncertainty associated with its use.

### *Motility*

Due to the heterogeneous nature of semen, estimation of uncertainty for motility is potentially difficult. Sperm motility is susceptible to fluctuations in temperature and starts to decline from the moment of ejaculation. This is where EQA distributions can be helpful when determining uncertainty. The use of recorded images ensures that all staff can assess the same sample, without any deterioration associated with a 'live' sample or other external influences.

However, other factors may add to the uncertainty of the motility assessment including the temperature of the sample when it is transported to the laboratory, the time from sample collection to examination and the temperature at which motility is assessed at.

Use of a heated microscope stage, when used correctly and independently verified to ensure the sample is assessed at 37 °C, is an effective way of ensuring that all samples are assessed at a constant temperature. However, it is the view of the Association of Biomedical Andrologists that the uncertainty associated with temperature of the sample is acceptable to within +/−1 °C, provided the temperature of the assessed sample never exceeds 37 °C. With an accepted uncertainty, this temperature range is considered critical and should therefore, be verified with a metrologically traceable thermometer.

### *Morphology*

Morphology assessment is often considered to be the most subjective part of the SA. The historic change to reference limits has also driven changes in the way sperm morphology is assessed. As morphology smears are routinely stained before analysis, these fixed samples can be used to determine uncertainty. To prevent fixed morphology slides from deteriorating, they should be mounted and stored in the dark. Repeat analysis of the same slide or group of slides allows an estimation of uncertainty to be made. This information should be used in conjunction with information obtained from EQA distributions, as without a target value there is no way of confirming if the method of training staff has introduced a systematic bias into the analysis.

### *Computer assisted semen analysis*

Where a laboratory elects to use an automated system as part of an SA, the laboratory must ensure that the system is able to provide at least the same level of accuracy as the manual methods. The laboratory should look to ensure that it is able to perform accurately across the full range of patient samples. The laboratory must determine the accuracy and reproducibility of the system for all parameters reported. No Computer Assisted Semen Analysis is an alternative to skilled and experienced andrology staff, however, in their hands they are a tool that has the potential to reduce the subjectivity within some elements of the analysis.

## Summary

Whilst, if is widely acknowledged that seminal fluid is a heterogeneous fluid, there are many opportunities to estimate measurement uncertainty. Information can be obtained from calibration certificates, EQA distributions, IQC and repeat analysis of patient samples. When obtaining data to estimate measurement uncertainty, the laboratory should ensure that the data covers the range of

values that the laboratory would expect to find and critically must include values around diagnostic thresholds. The analysis of these samples should be performed by all staff that perform the analyses. There must be a periodic review of the uncertainty and in addition uncertainty must be reassessed whenever changes occur to the analysis or there is a change of staffing.

The laboratory has the responsibility to implement and monitor practices that will reduce measurement uncertainty for the tests performed. Patients should be provided with clear instruction that explain what they must do prior to collecting their sample, how the sample should be collected, how the sample should be transported to the laboratory, to preserve the integrity of the sample. As part of the sample collection process, the laboratory should have a procedure to ensure that the patient has understood and complied with the instruction. It should be explained to the patient why it is important that the laboratory needs to ensure compliance. The laboratory has no option but to rely on patient honesty when determining the quality of the sample received, however, if the reasons are explained to the patient and that giving inaccurate information regarding sample quality may impact on their diagnosis the laboratory has done all they can. Compliance should be regularly reviewed, and if it is demonstrated that patients are unable to comply with transport and delivery requirements, the laboratory may need to decide if there is a requirement for sample production facilities on site.

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## ORCID

*D Sanders* http://orcid.org/0000-0001-8148-5318
*B Woodward* http://orcid.org/0000-0001-8669-4776

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