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## Article for *The Column*

**Reference materials – why quality matters**

Access to a comprehensive source of certified reference materials in various forms – powders and solutions – is vital for clinical and forensic laboratories, to ensure the correct identification of substances and the accuracy of results, and there is an increasing customer demand for ISO Guide 34:2009 certification. Jenny Button, Product Specialist, LGC Standards, UK, discusses certified reference materials and the importance of ISO Guide 34 to both manufacturers and end-users.

**What is ISO Guide 34?**

Laboratories must have confidence in the competence of the producer(s) from whom they obtain their reference materials, and manufacturers should be able to demonstrate this competence to regulatory authorities. ISO Guide 34 outlines the general requirements for the competence of reference material producers, and is used as a basis for the accreditation of manufacturers. Laboratories accredited to ISO/IEC 17025:2005 should use reference material providers that are accredited to ISO Guide 34.

**What does ISO/IEC 17025 say about use of reference materials?**

ISO/IEC 17025 outlines the general requirements for the competency of testing and calibration laboratories, setting requirements for measurement standards used in calibration, validation and quality control. Laboratories are expected to understand metrological traceability, comparability and uncertainty evaluation. The two latest editions of ISO/IEC 17025 put more emphasis on metrological traceability. This is reflected in sections 5.6.3.1 and 5.6.3.2, which say that *‘Measurement standards should be calibrated by a body that can provide traceability...’* and *‘Reference materials should, where possible, be traceable to SI units of measurements or to certified reference materials...’*. It is stressed that a testing laboratory must ensure that the calibrators used will enable the laboratory to meet the uncertainty requirement for the measurement (fitness for purpose). Using an ISO Guide 34 accredited producer ensures that the uncertainty measurement of the reference material has been calculated in accordance with ISO requirements.

**Does the same apply for ISO 15189 accredited laboratories?**

Yes. The ISO 15189:2007 standard is based on the details of ISO/IEC 17025, but takes into consideration the specific requirements of medical laboratories and their contribution to patient care, such as continuity of care, improved patient safety, risk mitigation and improved operational efficiency.

**How does ISO 9001 differ from ISO Guide 34, ISO/IEC 17025 and ISO 15189?**

ISO 9001:2008 is designed to assist organisations of all types and sizes in implementing and operating effective quality management systems. It is intentionally very generic. This accreditation is about providing a framework for continual improvement, and meeting customer requirements and expectations. It is different to, and should not be confused with, ISO/IEC 17025, ISO 15189 and ISO Guide 34, all of which incorporate the essential elements of ISO 9001, but add technical competency factors associated with their specific structure and function.

**What is the importance of traceability to the International System of Units?**

To be able to compare results over space and time, all individual results must be linked to some common metrological reference or measurement standard. These references, or ‘anchor points’, define what is correct. The **International System of Units (**Système International d’Unités, SI) is recognised worldwide as the ultimate authority in scientific measurement. The ideal end point (reference) of a traceability chain is, therefore, a definition of an SI unit. Laboratories, as well as producers of reference materials, must be able to demonstrate that there is a link to a metrological reference. Traceability directly back to the SI gives laboratories confidence that the materials are not only as described in the certificate of analysis, in terms of concentration and identity, but that they are consistent and comparable between laboratories. Although other definitions of the traceability of reference materials exist, they are less robust.

**Are two sources of ISO Guide 34 material needed to satisfy accreditation requirements?**

One source of ISO Guide 34 certified reference material is sufficient to satisfy accreditation requirements. However, the use of two independent sources produced in accordance with ISO Guide 34 provides an additional level of confidence to both user and assessor. The cost of the consumables, analyst and instrument time required to repeat failed assays significantly exceeds the cost of using two independent ISO Guide 34 certified reference materials.

**Do calibrators and quality controls need to come from separate sources?**

The requirements for independent sources of reference materials for calibration and control are discussed in several ISO guidelines. ISO/IEC Guide 99:2007, International Vocabulary of Metrology (VIM), section 5.13, note 6, states that *‘In a given measurement,**a given reference material can only be used for either calibration or quality assurance’*, while note 4 – following the definition of reference material adopted by the Reference Material Committee of ISO (ISO REMCO) in 2005 – says that *‘A reference material can only be used for a single purpose in a given measurement’*. ISO Guide 31:2000 – currently under revision – modifies this to read *‘A single reference material cannot be used for both calibration and validation in the same measurement procedure’.*

The United Kingdom Accreditation Service, UKAS, acknowledges that there are occasions when it is difficult – perhaps even impossible – to find two independent sources of reference materials. In these circumstances, they advise that ‘*If the same source/batch of material is used for both calibration and quality control, measures must be in place to ensure that the possibility of contamination is not only considered, but that measures are in place to detect this. Any bias in the reference material used for calibration or quality control must be detectable by the quality control measures in place in the laboratory’.*

**Are all reference materials accompanied by a certificate ‘certified reference materials’?**

Most products will be supplied with some form of documentation, which may be referred to as a certificate; however, as a general caveat, it does not necessarily follow that this fulfils ISO requirements for a certified reference material. To comply with ISO guidelines, certificates must be issued by a technically competent body, such as the National Metrology Institute (NMI), or an ISO Guide 34 accredited producer. The requirements for the description of reference materials and the contents of certificates and labels are specified by ISO Guide 31 and ISO 15194:2009.

Certified reference material certificates should comprise: name and address of the manufacturer; material identification, including batch/lot number; description of the material and its intended purpose; instructions for use; storage conditions; product certification, with a full description of the methods used and uncertainty; date of document issue; and expiry date of the material.

**What is the difference between a certified reference material and a chemical?**

ISO/IEC Guide 99:2007 (VIM) defines a certified reference material as a material of known homogeneity and stability, which is accompanied by documentation (certificate of analysis) issued by an authoritative body that provides one or more specified property values with their associated uncertainties and traceabilities, obtained using valid reference measurement procedures. In the laboratory, a certified reference material is typically classified as a pure substance used to prepare calibration solutions, or matrix material used to evaluate measurement bias. Certified reference materials have been demonstrated to be fit for their intended use in measurement.

Chemicals are typically reagent grade or synthetic precursors, and can occasionally be mistaken for reference materials. They may be accompanied by documentation listing the basic physical properties of the material, and this documentation may even be referred to as a certificate; however, the materials do not comply with ISO requirements for certification and should not be assumed to be certified reference materials. The purity of a chemical is usually given as a minimum concentration, for example ≥98 %. They should not be used for quantitative purposes 'as is', although they may be suitable for use as secondary reference materials providing the purity is verified by comparison to a certified reference material.

**Some reference materials, such as legal highs, can be difficult to source. In these circumstances, is it acceptable to purchase them from online head shops?**

Commercial delays in the provision of reference materials and associated costs can make purchasing 'reference materials' directly from legal high outlets seem an appealing choice. Although 'grey market sourcing' of materials and their conversion into certified reference materials is one route that can be used by commercial suppliers in the production of standards, this approach is fraught with problems.

The legal high market is unregulated, and published studies have shown that products do not necessarily contain what they claim to contain. Moreover, content has been shown to vary on repeat purchase of the same material. Manufacturers often claim high percentage purity, and initial GC-MS analysis may appear to agree. However, further examination by techniques such as NMR and CNO – which are not widely available in most laboratories – can tell a very different story and, at best, you may end up with a fraction of the product you purchased after purification. At worst, you may incorrectly identify or quantify your unknown compound. Furthermore, products sold as legal highs have been found to contain one or more controlled substances and, depending on each individual country’s legislation, possession of these products may be illegal, leaving you liable to prosecution irrespective of whether or not you have knowingly purchased a controlled drug.

**Why are certified reference materials available as both powders and solutions?**

For most applications, either a powder or a solution is suitable, and each has its own advantages and disadvantages. Many people prefer solutions, as this removes the laborious process of having to weigh the materials themselves, eliminating weighing errors and any in­house errors associated with correction for purity, salt or water content. As the solubility of the material has already been investigated, you can be confident that the compound has been dissolved in an appropriate solvent.

Manufacturers acknowledge that some reference materials can be expensive. Vials may contain quantities as low as 1 milligram, making a material more affordable, but this is impractical in terms of weighing for quantitative purposes. Accurately calibrated, lower concentration solutions are one way of providing high quality materials at affordable prices.

Despite the advantages of solutions, some people still prefer powders; they may be more suitable for certain applications – such as Fourier transform infrared spectroscopy (FTIR) – and are also more appropriate for drugs that are unstable in solution.

**Why are some certified reference materials sold as free bases and others as salts?**

Salts (hydrochloride, sodium, sulphate, etc) and hydrates (addition of water) help to stabilise or increase the solubility of the parent compound. Errors in quantitative analysis can occur through failure to correct for salts and hydrates, and this can be avoided by manufacturers correcting to the concentration of free acid/base in the solution. Corrections are required for some – but not all – powders, and this is achieved by using the known percentage of parent compound in the material to convert the actual weight into the weight of free acid/base.

**Author biography**

Jenny Button has worked in clinical and forensic toxicology for 15 years, initially in a university hospital setting and subsequently for the UK Government forensic laboratory. She joined LGC Standards in 2011, and draws heavily on her own experiences with quality accreditation to provide a practical and customer-focused approach to her position as Product Specialist and Technical Advisor. Jenny is actively involved with a number of professional societies relating to clinical and forensic toxicology, including participation on IATDMCT and LTG committees, and has also co-authored several peer-reviewed articles, focusing largely on the analysis of new emerging psychoactive drugs.

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