



guardians of drinking water quality

DRINKING WATER INSPECTORATE

Zone 2/F4, Ashdown House
123 Victoria Street, London, SW1E 6DE

Direct Line: 020 7944 5933
Enquiries: 020 7944 5956
Facsimile: 020 7944 5969

E-mail: michael.rouse@detr.gov.uk
DWI Website: <http://www.dwi.gov.uk>

26 January 2001

Information Letter 2/2001

To: Board Level Contacts of Water and Sewerage Companies and Water Companies in England and Wales

Dear Sir/Madam

WATER SUPPLY (WATER QUALITY) REGULATIONS 1989: GUIDELINES FOR CALIBRATION IN LABORATORIES

Purpose

1. In 2000 the Inspectorate contracted LGC (Teddington) Ltd to prepare guidance for laboratories on basic calibration of laboratory equipment and the preparation of standard solutions. This letter introduces the resulting "Guidelines for Calibration in Laboratories"; (the Guidelines), and explains how the Guidelines will be used by the Inspectorate and the implications for companies and laboratories. You should ensure that copies of the letter and the Guidelines are passed to all laboratories carrying out regulatory drinking water analysis for your water company.

Introduction

2. During 1999 and 2000 significant deficiencies in calibration practice were identified by the Inspectorate during compliance sample audit trails (vertical audits), audits of laboratories carrying out compliance analysis, audits of laboratories for approval to carry out *Cryptosporidium* analysis and audits of laboratories for appointment as approved laboratories for materials testing for the Committee on Chemicals and Materials of Construction for use in Public Water Supply and Swimming Pools (from 1 January 2001 the Committee on Products and Processes for Use in Public Water Supply).
3. The deficiencies identified have ranged across the whole spectrum of calibration, and one or more deficiencies were identified in the majority of laboratories audited. Valid analysis relies on calibration that is carried out correctly and is traceable to national and international standards. Failure by laboratories to implement satisfactory calibration practice could result in

enforcement action, suspension or termination of approval, or prosecution as appropriate.

The Guidelines

4. The Guidelines have been produced as a statement of the recommended minimum requirements for satisfactory calibration in laboratories carrying out analysis for the purpose of Parts IV, V and VI of the Water Supply (Water Quality) Regulations 1989 as amended (the 1989 Regulations). This includes compliance monitoring, *Cryptosporidium* monitoring for the purposes of Regulation 23B and materials testing for approval for use in accordance with Regulation 25. The Water Supply (Water Quality) Regulations 2000 (the 2000 Regulations) will fully replace the 1989 Regulations on 1 January 2004. Regulation 23B of the 1989 Regulations was replaced on 1 January 2001 by regulation 29 of the 2000 Regulations, all other relevant provisions of the 1989 Regulations are replaced on 1 January 2004. The Guidelines are also a statement of the recommended minimum requirements for satisfactory calibration in laboratories carrying out analysis for the purposes of the 2000 Regulations. Wherever possible reference is made in the Guidelines to the practices accepted by the United Kingdom Accreditation Service (UKAS). Frequencies of calibration given in the Guidelines are minima and irrespective of any statement in the Guidelines recalibration or checking of calibration must always be carried out immediately following any occurrence that may have affected the calibration status of any item of analytical equipment.
5. The Guidelines are intended for use by laboratories carrying out regulatory analysis, approved *Cryptosporidium* Analytical Laboratories, laboratories carrying out testing of materials for approval for use in contact with water, and by the Inspectorate when auditing analytical arrangements for the above purposes. Water companies and laboratories will be expected to be able to demonstrate that any alternative calibration practice adopted is equivalent to or better than that recommended.

Enquiries about this letter

6. The Guidelines and the guidance in this letter may not be sufficient in all situations that may arise. In cases of doubt, a company or laboratory should consult the Inspector to whom it has been allocated for the purposes of technical audit or Mr M Morgan, Inspector, Floor 2/E1, Ashdown House (Direct Line: 020 7944 5987 E-mail Malcolm_Morgan@detr.gov.uk).
7. Copies of this letter are being sent to Pamela Taylor, Chief Executive, Water UK; Rodney Anderson, Water Supply and Regulation Division, Department of the Environment, Transport and the Regions; Bob Macey, Environment Division, The National Assembly for Wales; Tim Hooton, Water Services Unit, Scottish Executive; Randal Scott, Drinking Water Inspectorate for Northern Ireland; Rowena Tye, Office of Water Services;

the United Kingdom Accreditation Service; and all approved *Cryptosporidium* Analytical Laboratories and materials testing laboratories.

8. Please acknowledge receipt of this letter using the enclosed slip and envelope.

Yours faithfully

Michael Rouse
Chief Inspector
Drinking Water Inspectorate

GUIDELINES FOR CALIBRATION IN LABORATORIES

December 2000

DWI 70/2/107

Prepared for the Drinking Water Inspectorate by LGC (Teddington) Ltd

Contents

Background

Mass (Weight)

2.1 Weights

2.2 Weighing machines

Temperature

3.1 General requirements for calibration of reference temperature measuring devices

3.2 Reference liquid-in-glass thermometers

3.3 Working liquid-in-glass thermometers

3.4 Thermocouples

Time

4.1 Reference standard

4.2 Calibration of real time and elapsed time

4.3 Storage and handling of equipment

Length

5.1 Size measurement of small objects (c. 10^{-6} m) by optical microscopy

Volume

6.1 Volumetric apparatus

6.2 Syringes

6.3 Preparation of calibration standard solutions

Calibration certificate

References

1. Background

These Guidelines have been produced as a statement of the recommended minimum requirements for satisfactory calibration in laboratories carrying out analysis for the purpose of Parts IV, V and VI of the Water Supply (Water Quality) Regulations 1989 as amended (the 1989 Regulations). This includes compliance monitoring, *Cryptosporidium* monitoring for the purposes of Regulation 23B and materials testing for approval for use in accordance with Regulation 25. The Water Supply (Water Quality) Regulations 2000 will fully replace the 1989 Regulations on 1 January 2004. Regulation 23B of the 1989 Regulations is replaced on 1 January 2001, all other relevant provisions are replaced on 1 January 2004. These Guidelines are also a statement of the recommended minimum requirements for satisfactory calibration in laboratories carrying out analysis for the purpose of these new Regulations.

Significant deficiencies in calibration practice have been identified by the Inspectorate during compliance sample audit trails (vertical audits), audits of laboratories carrying out compliance analysis, audits of laboratories for approval to carry out *Cryptosporidium* analysis and audits of laboratories for appointment as approved laboratories for materials testing for the Committee on Chemicals and Materials of Construction for use in Public Water Supply and Swimming Pools (from 1 January 2001 the Committee on Products and Processes for Use in Public Water Supply).

The Guidelines are intended for use by laboratories carrying out regulatory analysis, approved *Cryptosporidium* Analytical Laboratories, laboratories carrying out testing of materials for approval for use in contact with water, and by Inspectors regulating and auditing those laboratories. Laboratories will be expected to be able to demonstrate that any alternative calibration practice is equivalent to or better than that recommended.

Wherever possible reference is made to the practices accepted by the United Kingdom Accreditation Service (UKAS).

Note: In these Guidelines all references to accredited laboratories assumes accreditation to ISO 17025 (or UKAS M10), that the accreditation provider is either UKAS or an equivalent scheme recognised through mutual agreement as being equivalent to UKAS, and that the accredited scope of that laboratory includes the measurement (calibration or test) of interest. The onus is on the user of that laboratory to confirm that that is the case.

Reference to a calibration certificate implies a certificate meeting accredited calibration requirements to ISO 17025 as shown in section 7. In some cases lesser certificates will be acceptable such as those issued by the manufacturers of volumetric glassware indicating product compliance.

2. Mass (Weight)

Both the National Physical Laboratory¹ and UKAS² provide useful general advice on weighing and weights.

2.1 Weights

2.1.1 Classification of weights

A number of organisations have published systems for the classification of weights. The system used by the International Office of Legal Metrology (OIML)³ is shown in Table 1 and classifies weights in terms of construction, tolerance and uncertainty. Another system is that used by the American Society for Testing and Materials (ASTM)⁴, who classify weights in terms of density, surface area, surface finish, surface protection, magnetic properties, corrosion resistance and hardness.

Table 1: OIML classification of reference weights

Class	Description	Tolerance	Permitted uncertainty	Re-calibration frequency
E1	Integral stainless steel weights without markings or adjusting chamber	±0.5 ppm at 1kg	±1/3 x permitted tolerance	2 years
E2	Integral stainless steel weights without markings or adjusting chamber	±1.5 ppm at 1 kg	±1/3 x permitted tolerance	2 years
F1	Stainless steel weights which may have a screw knob protecting an adjustment cavity	±5 ppm at 1 kg	±1/5 x permitted tolerance	1 year
F2	Weights of plated brass	±15 ppm at 1 kg	±1/5 x permitted tolerance	1 year
M1	Weights of brass (which are not corroded or tarnished) or cast iron with a good quality painted finish	±50 ppm at 1 kg	±1/5 x permitted tolerance	1 year
M2	Weights of brass or cast iron ('Traders' Weights')	±200 ppm at 1 kg	±1/5 x permitted tolerance	1 year

2.1.2 Selection of weights

Weights must be chosen so that their uncertainty is within that permitted by the application, see Tables 1 and 2 for guidance.

2.1.3 Identification of weights

Calibrated items are usually uniquely marked in order to relate them to a certificate of calibration and so maintain a chain of traceability. In the case of analytical balance weights, the normal practice is to mark the case in which the weights are supplied and stored, rather than the weights themselves.

Thus when two or more similar sets of weights are held by a laboratory care must be taken to ensure that weights are not mixed up between sets. Weights must be restored to their case as soon as possible after use.

If a weight is large enough, *i.e.* >1g, it may be inscribed with its nominal weight. In certain circumstances particular weight sizes may be duplicated within a set. For example it is not uncommon to have a series 50g, 20g, 10g, 10g, 5g, 2g, 1g, 1g, thus enabling a compound weight of anything between 1g and 99g. Given that each weight will have its own uncertainty, it is important to be able to distinguish between weights of the same nominal size. Ideally in such circumstances the weights will have distinguishing marks added by the manufacturer, prior to calibration. They may only be marked subsequently using a method that does not change the state of calibration, which is very difficult to guarantee. In the case of small weights made from wire or foil, which cannot be marked, it may be possible to distinguish them in terms of shape.

2.1.4 Storage and handling of weights

If a laboratory buys a set of weights, specified as being of a particular class, from a reputable supplier, it may assume that the construction of the weights is in keeping with the requirements of that class. This is a particularly reasonable assumption if the weights are also supplied with a calibration certificate from an accredited laboratory.

Weights will normally be supplied in a dedicated storage case and should be kept in this case whenever they are not in use. This keeps them in the optimum condition and minimises the risk of mixing them up. They must be stored in a clean, dry environment, away from chemical contamination. The higher grades of weights are designed to have a low magnetic susceptibility, but must be kept away from electric or magnetic fields, which could cause them to become magnetised.

Before use, weights must be equilibrated with the laboratory environment in which they are to be used.

It is difficult to clean weights without compromising their calibration. If weights are stored and handled correctly they should not become dirty. Electrostatic

contamination with dust, which is difficult to avoid, can be removed using a light stream of clean, dry, dust-free air or gentle brushing with a camel hair brush.

Class E and F weights must never be handled with bare hands. Clean chamois leather or lint-free cotton gloves should be worn. Small weights should be handled with tweezers tipped with plastic or ivory, or made from soft metal such as brass or copper.

Avoid knocking weights together or sliding them across abrasive surfaces. Any surface on which a weight is placed, including the weighing pan of the balance, must be clean and dry.

2.1.5 Calibration of weights – general

Table 1 shows, for each class of weights, the maximum uncertainty allowable in the calibration as a function of the stated tolerance, and the re-calibration interval. The American Society for Testing and Materials (ASTM) lists similar tolerances for non-metric weights⁴.

For all weights, the change in mass allowable between successive calibrations shall be less than the uncertainty of calibration as stated on the calibration certificate. Because of this it is likely that a frequently used weight will require more frequent calibration than a weight used infrequently.

2.1.6 Calibration of reference weights⁵

Reference weights must be calibrated or recalibrated either:

- a) by NPL or a National Standards Laboratory recognised by NPL (E1, E2, F1 weights), or;
- b) by a UKAS accredited laboratory or equivalent (F1, F2, M1, M2 weights).

2.1.7 Calibration of secondary weights

Non-reference weights may be calibrated at NPL, a UKAS accredited laboratory, or in-house using suitable (UKAS accredited) documented procedures, either using calibrated reference weights or a suitably calibrated weighing machine⁶.

In each case the calibration must show appropriate traceability.

2.1.8 Calibration records

Proof of calibration shall be in the form of a suitable certificate, showing quality marks where applicable. In the case of in-house calibration a full record of the calibration data, including traceability and uncertainty shall be available, together with a record of the procedure used. Certificate or record shall reference any unique identification on the weights.

2.2 Weighing machines

2.2.1 Calibration of weighing machines

Weighing machines shall be formally calibrated at regular intervals across their working range or exceptionally over a limited working range. Calibration status shall be evident on the machine, preferably through some form of labelling. Machines out of service, either because of lapsed calibration, or other reasons, must be labelled as such and must not be used. The frequency of calibration will depend on use but must be at least once a year. Re-calibration shall take place following major changes to the operating environment, movement of the machine and following service or repairs.

Due to the effect of instrument movement on calibration all calibrations must be performed in-situ, either by the laboratory itself, or by a suitably qualified (*i.e.* accredited for site work) agent.

Calibration shall be by external means only. The use of internal calibration procedures tend to adjust the balance and therefore negate any prior external calibration. Use of such routines is therefore discouraged unless it can be proved that such internal procedures do not break the chain of traceability.

2.2.2 Procedures for calibrations

NIS 6 gives examples of in-house calibration procedures for electronic balances and two-pan balances⁵. These examples are provided for guidance and illustration. The errors quoted in these examples are not to be taken as recommendations. The ASTM also list methods for evaluating various types of mechanical balance^{7,8,9}. Calibration procedures are available from a wide variety of other sources.

The following parameters must be tested: repeatability; sensitivity; departure from nominal value, off-centre loading, effect of tare mechanism.

Weights used in the calibration of weighing machines must be appropriate to the accuracy and range of the machine. The uncertainty associated with the weights must be less than the resolution of the machine. Table 2 indicates which types of weights are suitable for the calibration of various types of balance.

2.2.3 Checks using routine check weights

Between full calibrations, periodic performance checks shall be made. Each day that a balance is used a routine check weighing must be carried out. If the machine is normally used for a small range of weighings a single check, using a typical weight, is sufficient. If a wide range of weighings is normally made several weights spanning the range must be used. In either case, the weights used may either be calibrated weights, taken from the calibration set, or weights or artefacts kept specifically for checking purposes and calibrated by weighing, immediately following the last full calibration of the machine. The

checks shall include a zero check or adjustment, and weighing. The size of weights used should be typical of the weighings made, particularly if sub-100g weighings are made.

Accumulated data from the check weighings can be plotted to detect drift or step changes in the performance of the machine.

The measurement made with the check weight must be compared with a defined action limit or error allowance. If the weighing falls outside of the limit, a suitable course of action shall be taken and recorded. If the problem persists, the machine must be withdrawn from service, labelled as "out-of-service", appropriate remedial action taken and a full calibration undertaken before the machine is returned to service.

Table 2 Lowest class of weights required for different types of weighing machines

	Resolution							
	100g	10g	1g	100mg	10mg	1mg	0.1mg	1.£0.01 mg
Capacity								
Up to 200g				M1	M1	F2	F1	E2
200g to 1kg			M1	M1	F2	F1/E2	E2	E2
1kg to 30kg	M2	M2	M1	F2	E2	E2	E2	
30kg to 100kg	M2	M1	F2	F1	E2			
Above 100kg	M2	M1/F2	F1	E2				

3. Temperature

This section, based on UKAS NIS 7¹⁰, covers temperature measurement using the following devices:

- Liquid-in-glass thermometers
- Thermocouples
- Platinum resistance thermometers
- Radiation thermometers

It is recommended that a laboratory has a selection of working devices for everyday use. These must be traceable to national/international temperature

standards by means of calibration against a suitable reference device, whose role is restricted to calibration purposes only.

3.1 General requirements for calibration of reference temperature measuring devices

All reference devices must be calibrated either by the National Physical Laboratory (NPL), or equivalent, or by a UKAS accredited calibration laboratory, or equivalent. A valid certificate must be held, consistent with Section 7, as proof that a satisfactory calibration has taken place.

Traceability of the calibration must be to the appropriate International Temperature Scale.

All reference devices must undergo full calibration/re-calibration at suitable intervals, which may depend on use and storage but shall be determined as less than the time taken for there to be any significant change in accuracy and must not exceed the maximum intervals given in Table 3, below. In addition, liquid-in-glass thermometers and platinum resistance thermometers require periodic checking of reference points.

Before calibration the device must be inspected for damage and the calibration shall proceed only if the device is undamaged, e.g. in the case of thermometers, cracks in the fabric of the thermometer, or breaks in the mercury or alcohol thread would constitute damage.

Table 3 Calibration intervals for reference temperature measurement devices

Type	Maximum re-calibration interval	Reference point check	Maximum reference point check interval
Liquid-in-glass thermometers	5 years	Ice point or other appropriate point	12 months
Thermocouples	4 years ¹¹		
Platinum resistance thermometers	5 years ¹²	Ice or water triple point	Before each use
Radiation thermometers	2 years ¹³		

3.2 Reference liquid-in-glass thermometers

3.2.1 Selection

Reference liquid-in-glass thermometers must be selected according to the precision and temperature range demanded by the corresponding working thermometers. A wide range of British Standard and International Standard specifications are available^{14,15,16,17,18,19,20}.

Selection should also account for the use to which the thermometer is to be put. For example, temperature monitoring of refrigerators, freezers and incubators is required principally to ensure that temperature is maintained between maximum and minimum limits. Single daily readings of a conventional thermometer give little assurance of the extremes of temperature achieved between the readings. The preference in this case is to use a max/min thermometer calibrated at the required extremes or an electronic probe programmed to show maximum and minimum temperatures with the option of an alarm facility.

3.2.2 Calibration of reference liquid-in-glass thermometers

Calibration of reference liquid-in-glass thermometers must be carried out at least once every five years.

In addition to full re-calibration, the ice-point (or some other suitable reference point) shall be checked at least annually. These reference checks may be carried out by a UKAS accredited laboratory or in-house. A suitable method for checking the ice-point is given in BS 1041:Part 2²¹.

3.3 Working liquid-in-glass thermometers

Where the accuracy of a temperature measurement has a significant effect on a test result or its uncertainty a calibrated thermometer must be used for the measurement, calibrated either by NPL or a UKAS accredited calibration laboratory or equivalent and a valid certificate held. Alternatively it may be calibrated in house against one or more reference thermometers held by the laboratory. Guidance on calibration procedures may be found in BS 1041:Part 2²¹.

A new thermometer may require more frequent checks due to significant changes in calibration as the glass bulb volume stabilises. It is recommended that the reference point be checked every 6 months in the first year.

The frequency of re-calibration shall follow the same rules as for reference thermometers. In addition to full re-calibration, the ice-point (or some other suitable reference point) must be checked at least annually (but see note above for new thermometers). These reference checks may be carried out by a UKAS accredited laboratory or equivalent, or in-house. A suitable method for checking the ice-point is given in BS 1041:Part 2²¹. If a reference point check indicates a significant change in the thermometer, e.g. a shift of $>0.1^{\circ}\text{C}$, then it must be re-calibrated.

Care should be taken to ensure that thermometers are used in the manner intended. Thermometers may be specified as total- or partial immersion. Total immersion thermometers will give an accurate reading when used in the open air. A partial immersion thermometer is intended to be used by standing the bulb in the correct depth of water or other suitable liquid. Thus in a refrigerator, a partial immersion thermometer could be used by standing it in a

beaker within the refrigerator. The uncertainty of measurement of the thermometer used must be less than the permitted temperature range.

If the temperature range, within which a refrigerator, freezer or incubator operates, needs to be monitored it must be done on a continuous basis. There is a danger with, for example, single daily measurements, that the instrument may cycle out of range and back in again during the intervening period, without any indication that the temperature has been other than constant. It is more appropriate therefore to monitor the temperature, either by use of a max/min thermometer of a suitable range, or an electronic probe. In the case of the former, readings should still be taken at regular intervals and the pins will need to be reset with a magnet after each set of readings. The electronic probe can often be set up to give a continuous readout, perhaps using a chart recorder and may also be programmed to sound an alarm if the measured temperature falls outside of the specified range. Dual temperature incubators *etc.* will therefore require monitoring with electronic probes to establish that the incubator cycled correctly and operated within permitted tolerances at both temperatures.

3.4 Thermocouples

3.4.1 Calibration of reference thermocouples

The re-calibration interval given in Table 3 is the absolute maximum. The required interval becomes shorter, the closer the actual working temperature is to the recommended maximum working temperature for the particular thermocouple, as listed in BS 1041:Part 4:1992¹¹. Calibration must cover the full range of temperatures of interest.

3.4.2 Calibration of working thermocouples

Working thermocouples must either be calibrated in-house against a reference thermocouple or reference thermometer. The recalibration interval will depend on the normal working temperature, whether the temperature is fixed or variable and whether the thermocouple is used continually or intermittently. The interval should not exceed one year. Calibration must cover the full range of temperatures of interest. The uncertainty of measurement of the thermocouple used must be less than the permitted temperature range.

4. Time

4.1 Reference standard

It is unusual to have a reference and working timer system within a single laboratory. The generally accepted practice is that all timers used in a laboratory are considered as working timers. These are calibrated, for both real and elapsed time, by comparison against an external reference standard, namely the "speaking" clock or the Rugby "Atomic" time signal.

4.2 Calibration of real time and elapsed time

Calibration of timers for real time measurements is performed simply by ringing up the "speaking" clock and setting the timer to the time given. Real time calibration should be carried out at least twice a year, immediately following the spring and autumn change of reference point.

Calibration of timers for elapsed time is performed by taking two "readouts" from the "speaking" clock, spaced by a suitably large interval. A significant contribution to the uncertainty in the calibration arises from the human reaction times during the making of the comparison. In order to minimise the effect of this contribution, the time interval used for the comparison must be as large as possible, *e.g.* at least one hour. Elapsed time calibration should be carried out at least once a year.

In the case of clocks or watches, which link to the atomic clock at Rugby, these timepieces re-adjust themselves typically every two hours against the Rugby time signal. Although theoretically the same time intervals would vary if taken within or across a two-hourly adjustment, the difference will be negligible for most purposes and probably less than uncertainties arising from the use of the speaking clock as a reference.

4.3 Storage and handling of equipment

Mechanical timers will probably be more susceptible to changes in the laboratory environment than electronic timers or those regulated by a quartz movement. As a general rule timers should not be subjected to any extremes of temperature or humidity. They should also be shielded from exposure to mechanical shock, dust, and strong magnetic or electrical fields, and immersion in liquids.

5. Length

5.1 Size measurement of small objects (c. 10^{-6} m) by optical microscopy

Microscopy is used in water analysis to detect, identify and count small particles in water supplies. These small particles may include microbial organisms which are typically detected according to size, colour, appearance, and behaviour in the presence of specific visualisation techniques. It is important to be able to size particles accurately.

Typically image size is characterised using some form of calibrated microscopic scale, sometimes in the eyepiece but usually on the microscope stage. These scales are called Graticules or eyepiece micrometers or stage micrometers. They are available in a number of patterns and sizes for a diverse range of applications.

5.1.1 Identification

Reference Graticules and stage/eyepiece micrometers must be uniquely identified by marking on the slide frame itself. This enables traceability to the corresponding calibration certificate.

5.1.2 Calibration

As a general rule the calibration of reference graticules and stage/eyepiece micrometers is a highly specialist operation and needs to be performed by a dedicated laboratory with appropriate UKAS accreditation and traceability to a national standard, e.g. through the National Physical Laboratory.

5.1.3 Handling and storage of Graticules and stage micrometers

Reference Graticules and stage/eyepiece micrometers must be kept clean and stored in their dedicated packaging when not in use. They must not be subjected to extremes of temperature, which may cause irreversible distortion which may invalidate their calibration. With careful use 5 years is considered a reasonable recalibration interval.

Graticules and stage/eyepiece micrometers are fragile. When in use they must be handled with care, in accordance with the makers instructions, and avoiding any contact with the optical faces which may result in damage or contamination.

Handled carefully Graticules and stage/eyepiece micrometers should not get dirty, but if any cleaning is necessary it should be done in accordance with the manufacturers instructions.

5.1.4 Calibration of working eye-piece Graticules

Recalibration of working eye-piece Graticules against the reference stage micrometer is recommended at regular intervals (monthly) and following maintenance, repair or relocation of the microscope. A full record must be made of each calibration.

In the particular case of regulatory monitoring for *Cryptosporidium* oocysts, the calibration must be checked on each day that a preliminary result of 0.5 oocysts or greater in 10 litres is encountered when using that microscope. The calibration of each microscope used for either internal or external confirmation of results must be checked before use on each day that the microscope is used for that purpose. A full record must be made of each calibration check.

6. Volume

Accurate and precise measurement of volume is a key factor in quantification in chemistry. Volumetric apparatus of various types consequently plays a very important role²².

6.1 Volumetric apparatus

Volumetric apparatus either contains or delivers a well-defined volume of liquid. Traditionally it has been made from glass. Increasingly it also includes items made of various plastics. Each type may be further sub-divided according to whether it is for a single volume (single mark) or for multiple volumes (multi mark). The use of auto-pipettes (single and adjustable volume), automated burettes and auto-dispensers is becoming increasingly popular, mainly for reasons of convenience and especially where there is a high degree of repetition. Syringes, similar in their principle of operation to auto-pipettes, are dealt with in a separate section.

Most items of volumetric glassware are available commercially in two grades: Class A (formerly grade A) and Class B (formerly grade B). The distinction between the two is essentially one of tolerance. For a given volume, the tolerance for Class B is twice that for Class A. The specification for various types of volumetric glassware including tolerances and marking is laid down in the relevant British or international standards^{23,24,25,26,27}. Similar specifications are available for volumetric plasticware^{28,29}. Suppliers sell glassware as conforming to either Class A or Class B in accordance with a particular published standard. Certificated glassware is usually an option at higher cost. Certification may come in one of two forms. A *certificate of conformance* is proof that the item complies with the tolerance indicated by the particular standard. A *certificate of calibration* (compliant with UKAS's requirements) should actually state an absolute volume with corresponding uncertainty and indicate the chain of traceability. For both types of certificate the related item should be uniquely marked to ensure traceability to its certificate. The onus is on the purchaser, when buying glassware, to ensure that any calibration or certification will meet their needs.

Opinion is divided on the relative merits of using traditional glass pipettes and auto-pipettes. It is worth remembering when comparing the two that published glassware tolerances do not take into account errors related to human use. The fairest comparison is to determine the accuracy and precision of each by weighing multiple (e.g. 10) discharges of water.

The laboratory shall be responsible for ensuring that the glassware used is of a class appropriate for the application and that the items used actually comply with the stated class. Class A need only be used where such volumetric accuracy is critical to the performance of the method. In such cases the glassware must be calibrated as conforming to Class A tolerances and proof provided, either in the form of a certificate, or in the case of in-house calibration, a record of the calibration data.

Where a test method specifies accuracy and precision consistent with the use of Class A tolerances but does not specify the use of calibrated Class A glassware, DWI reserves the right to require that calibrated Class A glassware be used.

6.1.1 Marking

Volumetric glassware shall be marked in accordance with standard requirements. A number of British standards and the international equivalents specify this marking, which shall include:

- tolerance as 'Class A' or 'Class B';
- a mark to distinguish whether it is made from soda-lime or boro-silicate glass;
- capacity in 'ml', 'mL' or 'cm³';
- reference temperature (i.e. temperature at which calibrated, usually 20° C);
- identification number (to uniquely cross reference the item to the corresponding calibration certificate);
- time of flow / delivery time (for burettes and pipettes);
- maker's mark;
- colour coding (in accordance with BS 3996³⁰).

6.1.2 Use

In order to get the optimum performance from volumetric apparatus, it is important to use it in the way intended.

Pipettes

Unless it is of the blow-out type a pipette should be allowed to drain naturally. For water based solutions, the specified volume is deemed to have been delivered after the waiting time quoted on the item. More viscous liquids will require a longer draining time, less viscous liquids may over-deliver in the quoted time. Damage to the tip of a pipette may affect its draining characteristics and therefore its calibration.

When using graduated pipettes the pipette should always be filled and allowed to drain in accordance with the manufacturer's instructions. Some are calibrated from the zero mark (fill to the zero mark and then allowed to discharge the desired volume); other are calibrated from the tip (fill to the desired volume and allow to drain fully). Similar principles apply to the use of burettes.

Filling pipettes by sucking using the mouth is inadvisable for safety reasons.

Auto-pipettes, auto-titrators, auto-dispensers.

Follow manufacturers' instructions carefully in order to get optimum performance. Avoid contamination through drawing liquid into the main body

of the instrument. In the case of adjustable volume pipettes and dispensers it is good practice to dial up the required volume from the same direction to compensate for any play in the screw thread mechanism.

6.1.3 Use for storing solutions

Volumetric glassware is not intended for the long-term storage of liquids or solutions. It is relatively expensive and its use for storage ties up valuable resources. Volumetric flasks are also relatively fragile. Stored solutions are often refrigerated and it is preferable not to expose volumetric glassware to temperature fluctuations lest this upsets its calibration. If a liquid is stored in a volumetric flask for any period of time it is recommended that plastic stoppers are used in case there is any seepage, particularly of alkali or crystalline solutions which may cause glass stoppers to jam.

6.1.4 Cleaning

Care should be taken when cleaning volumetric glassware. Detergents should be used that do not cause the inner surface of the glassware to become activated. This is particularly important where the operation of the glassware depends on surface tension, e.g. pipette draining times.

Ideally, once clean, glassware should be rinsed with de-mineralised water and allowed to dry naturally by draining. Heat drying should be avoided as glass tends to suffer non-elastic expansion when heated thus putting the glassware out of calibration.

6.1.5 Temperature operating limits for volumetric glassware

Volumetric glassware is normally calibrated at 20° C (27°C in tropical countries). Use of volumetric solutions at different temperatures may result in significant errors. Table 4 shows the temperature limits at which volumetric apparatus of various volumes fall outside their Class A tolerances.

Table 4 Temperature limits at which Class A glassware exceeds tolerances

Volume /ml	Temperature Limits / °C
10	<31
50	10-26
100	13-25
250	16-23
1000	17-22

6.1.6 Re-calibration of volumetric apparatus

Glassware

Depending on how it is used, volumetric glassware may require re-calibration after a year or less. Re-calibration, if required, can be performed in-house using gravimetry. Procedures for the calibration of volumetric glassware are given in BS 6696³¹. However, it may be more convenient and economic to replace the glassware with new certified stock and relegating the out-of-calibration stock to non-critical purposes.

Note that, unless specified otherwise, calibration data for glassware relates to calibration using water at a particular temperature. Use of volumetric glassware for solvents other than water should not rely on the water calibration. Where critical, the calibration must be repeated, using the appropriate solvent.

Auto-pipettes

Standard methods are available for calibration of mechanical hand held pipettes^{32,33}. Laboratories shall make regular (*i.e.* each day that the item is in use) checks for gross errors by weighing successive discharges into a beaker. Manufacturers should give guidance on recalibration periods, and periodic performance checks should indicate when recalibration or other maintenance is necessary. In the absence of evidence or advice to the contrary, 12 months is a reasonable interval with normal use.

For variable volume pipettes the volume should always be set from the same direction, in case of any play in the adjustment mechanism.

Auto-dispensers work on a similar principle to automatic pipettes and the same rules apply. Check valves enable a pre-set volume of the liquid of interest to be drawn up from an integral reservoir and then discharged through a separate tube into the receiving vessel.

6.2 Syringes

These are specialist volumetric instruments usually designed for filling and emptying via a needle, which may be fixed or detachable. Syringes fall into two broad categories: microsyringes and disposable syringes.

6.2.1 Microsyringes

These are high precision metal and glass instruments, often designed for a specific application and with claimed accuracy and precision of around 1%. They are used for various applications, one of the most important being sample introduction in certain analytical methods and instruments. They may also be used as pipettes for dispensing small volumes.

- i) *Sample introduction via a septum.* In general the same syringe will be used for sample, blank and standard, so accuracy is not really a concern although precision will be. A pointed needle is the preferred type. In practice imprecision will be evident through variations in chromatographic peak size, but this will be due to other factors besides the syringe

precision. Where the samples already contain an internal standard precision is not an issue. Observed variations in accuracy or precision greater than those expected may adversely affect the uncertainty of results, and could indicate a fault in either the microsyringe, the septum or another component of the analytical system. All such occurrences must be investigated immediately and corrective action taken before proceeding.

- ii) *Sample introduction via a valve.* This method utilises a loop which is overfilled from the syringe (typically 1ml capacity) so the precision of the syringe is not important. The needle should be flat topped (so as not to damage the valve) and of the appropriate length (to prevent blow-back).
- iii) *Thin layer chromatography sample loading.* The syringe is used to dispense the sample onto the TLC plate in small amounts, to prevent the sample spot spreading unduly. A flat ended needle is preferred to minimise damage to the TLC plate surface. Accuracy and precision are both important in this application.
- iv) *Use as a pipette.* A flat ended needle is preferred. Accuracy and precision are both important in this application.

Calibration of microsyringes

The nominal volume of microsyringes may be as low as 1 μ l, so the weight of 1% of 1 μ l will be around 10 μ g! Accurate weighing at this level is far from straightforward so gravimetry is not really a practical way of calibrating. As a general rule measurements which involve differences of less than 1mg shall be justified or avoided.

Theoretically an alternative approach is to use colorimetry using a strongly absorbing liquid and a good quality double-beam UV-Visible spectrophotometer, with well matched cells. Use the syringe to dilute the liquid so that the dilution has an absorption of approx 1.000. The resolution of this technique is therefore about 0.1% (\approx 0.001 absorbance units). If the absorption coefficient of the liquid is well characterised the method can determine accuracy as well as precision. The flask used for the dilution will also need to be accurately calibrated.

Microsyringes should be recalibrated whenever there is any evidence that either accuracy or precision has changed, if the characteristic that may have changed is of importance to the application.

6.2.2 Disposable syringes

These are often disposable, general purpose plastic syringes with relatively low volumetric accuracy, of the order of 5%. Capacity tends to be at least 1cm³ and so gravimetry is a practical means of calibration.

6.3 Preparation of calibration standard solutions

Operating procedures shall be designed to preserve the integrity of the master standard or reagent. This shall include avoidance of any sampling instrument (*i.e.* pipettes, spatulas, sample vials, *etc.*) coming into direct contact with, and causing possible contamination of, the bulk.

6.3.1 Sub-sampling

In the case of the master standard or reagent being either a liquid or solution, sub-sampling shall be achieved by decanting a small volume, sufficient for the subsample(s), into a suitable clean vessel, and portions removed from this for the purposes of further dilution.

Likewise, where the master standard or reagent is a solid, sufficient shall be transferred to a suitable vessel, and portions removed from this for further use.

Whether liquid or solid, on no account must any residual master standard or reagent be returned to the bulk at the end of the exercise.

Where it is necessary to flush sampling instruments with the standard or reagent prior to sub-sampling, on no account must the flushings be returned to either the bulk or the sub sample of standard or reagent.

6.3.2 Single or multiple stage dilution?

Often when making a dilution there are a number of different ways it can be done. Should it be done in one stage or several? A number of factors need to be considered:

What is the allowable uncertainty contribution from the dilution stage?
Relative tolerances are generally higher on smaller volumes, so use of small volumes leads to higher uncertainties. Although uncertainties from autopipettes appear generally higher than those for glass pipettes for the same volume, use of the latter involves further uncertainties relating to human errors. This comparison has been discussed above.

How expensive is the sample or reagent that is to be diluted - and how much is available?

How expensive is the diluent?

What volume of the final dilution is required?

Are there any particular problems associated with disposal of large quantities of final solution or diluent?

Table 5 shows six different ways of making a 200X dilution, and the associated uncertainty from the volumetric tolerance. It has been estimated

that volumetric tolerance accounts for only about 20% of the total uncertainty of a volumetric stage.

Table 5 Different ways of making a 200X dilution

	200 X dilution	Initial volume diluted / ml	Final volume of dilution / ml	Volume of diluent used / ml	Accumulated uncertainty arising only from volumetric tolerances * / %
1	1ml→200ml	1	200	199	0.93
2	5ml →1000ml	5	1000	995	0.35
3	5ml→100ml, 10ml →100ml	5	100	185	0.45
4	10ml→100ml, 10ml→200ml	10	200	280	0.36
5	100ml→1000ml, 50ml→1000ml	100	1000	1850	0.16
6	100ml→500ml 50ml→200ml 100ml→1000ml	100	1000	1450	0.20

* Uncertainties are for class A glassware stated at 95 % confidence (k=2). Taking the specified tolerance of the relevant item of glassware, the tolerance is assumed to be a rectangular distribution and the consequent uncertainty is obtained by dividing by $\sqrt{3}$. The accumulated uncertainty for each method of dilution is obtained as the square root of the sum of the squares of the uncertainty contribution for each item of glassware involved in the dilution. The accumulated uncertainty is then multiplied by 2 to convert it to the uncertainty at 95% confidence.

Although uncertainties arising from any volumetric stage should be considered their contribution to the overall uncertainty of the measurement will not be significant if there are other, dominating uncertainties. For example, uncertainty arising from the completeness of an extraction stage may easily be well in excess of 20%. Table 6 illustrates the effects of various volumetric uncertainties on an extraction uncertainty of 20%, and Table 6a the effects of the same volumetric uncertainties if the extraction uncertainty were 5%. The uncertainties have been combined by the root sum of squares method:

Table 6 Effect of various volumetric uncertainties on a dominant extraction uncertainty

$U_{\text{extraction}} / \%$	20	20	20	20	20
$U_{\text{volumetric}} / \% >$	1	2	5	10	20
$U_{\text{Total}} / \%$	20.02	20.1	20.6	22.4	28.3

Table 6a Effect of various volumetric uncertainties on a % extraction uncertainty

$U_{\text{extraction}} / \%$	5	5	5	5	5
$U_{\text{volumetric}} / \% >$	1	2	5	10	20
$U_{\text{Total}} / \%$	5.1	5.4	7.1	11.2	20.6

7. Calibration certificate

ISO 17025 specifies the following minimum information for a calibration certificate:

- Title
- Name and address of measurement laboratory performing the calibration and/or location where the calibration was carried out
- Unique identification of the certificate and information on each page to show it to be part of the certificate and that the certificate is complete.
- Name and address of the client.
- Identification of the method used.
- Description of the condition of, and unambiguous identification of the item calibrated.
- The date of receipt of the calibration item where this is critical to the validity and application of the results and the date(s) of the performance of the calibration. The re-calibration interval should not be quoted unless this has been agreed with the client
- Reference to any sampling plan and procedures used where relevant to the results.
- Results with units as appropriate. When an item has been adjusted or repaired the calibration results before and after adjustment/repair shall be reported.
- Name, function and signature of the person authorising the certificate.

- Statement to the effect that the results relate only to the items calibrated.
- The conditions under which the calibration was made which might influence the results.
- Statements of uncertainty of measurement and/or compliance with an identified metrological specification.
- Evidence that the calibration is traceable.

References

1. Guide to the Measurement of Mass and Weight, 1998, Published jointly by NPL and the Institute of Measurement and Control, ISBN 0 904457 27 3.
2. UKAS NIS 0416:1990: Guidance on Weighing in NAMAS Accredited Laboratories.
3. OIML reference for weight classification.
4. ASTM E617-91. Specification for Laboratory Weights and precision mass standards.
5. UKAS NIS 6:1992: Calibration of Weighing Machines and Weights.
6. Ref for in-house procedure if available.
7. ASTM E319-93, Standard Test Method for the evaluation of single-pan mechanical balances.
8. ASTM E898-88: Standard Method for Testing Balances Lab scales - top-loading - direct reading.
9. ASTM E-1270 Standard method for testing equal arm balances
10. UKAS NIS 7:1995: Traceability of Temperature Measurement: Liquid-in-glass Thermometers, Thermocouples, Platinum Resistance Thermometers and Radiation Thermometers.
11. BS 1041:Part 4:1992, Guide to the Selection and Use of Thermocouples.
12. BS 1041:Part 3:1989, Guide to the Selection and Use of Industrial Resistance Thermometers.
13. BS 1041:Part 5:1989, Guide to the Selection and Use of Radiation Pyrometers.

14. BS 593:1989, Specification for Laboratory Thermometers.
[¹ISO 386:1977, Liquid - in-glass laboratory thermometers - Principles of design, construction and use].
15. BS 1900:1976(1992), Specification for Secondary Reference Thermometers.
16. BS 5074:1974(1992), Specification for Short and Long Stem Thermometers for Precision Use.
[= ISO 653:1980, Long solid-stem thermometers for precision use, = 654:1980, Short solid-stem thermometers for precision use, ¹ISO 386:1977, Liquid - in-glass laboratory thermometers - Principles of design, construction and use].
17. BS 1365:1990(1997), Specification for short-range short-stem thermometers.
18. BS 1704:1985(1992), Specification for solid-stem general purpose thermometers. [⁰ ISO 1770:1981, Solid-stem general purpose thermometers].
19. ISO 655:1980, Long enclosed-scale thermometers for precision use.
20. ISO 1771:1981 Enclosed scale general purpose thermometers (with amendment 1:1983).
21. BS 1041:Part 2.1:1985(1992), Guide to the Selection and Use of Liquid-in-glass Thermometers.
22. UKAS NIS 5:1995: Traceability: Volumetric Apparatus.
23. ISO 648:1977, Laboratory glassware - One-mark pipettes.
24. ISO 835-1:1981, Laboratory glassware - Graduated pipettes - Part 1: General requirements.
25. ISO 835-2:1981, Laboratory glassware - Graduated pipettes - Part 2: Pipettes for which no waiting time is specified.
26. ISO 835-3:1981, Laboratory glassware - Graduated pipettes - Part 3: Pipettes for which a waiting time of 15 seconds is specified.
27. ISO 835-4:1981, Laboratory glassware - Graduated pipettes - Part 4: Blow-out pipettes.
28. ISO 6706:1981, Plastics laboratory ware – Graduated measuring cylinders.
29. ISO 12771:1997, Plastics laboratory ware – Disposable serological pipettes.

30. BS 3996:1978(1991), Specification for colour coding of one-mark and graduated pipettes (including requirements for the service performance of the colour coding enamels) Equivalent to but not identical to ISO 1769:1975, ISO 4794:1982.
31. BS 6696:1986(1992), Methods for use and testing of capacity of volumetric glassware. [186; ISO 4787:1984]
32. BS 6018:1991, Method of test for accuracy and precision of mechanical hand pipettes of capacity 0.05 ml and above.
33. BS 7532:1991, Method of test for accuracy and precision of mechanical hand pipettes of capacity less than 50 μ l down to and including 10 μ l.

All the references listed are extant at the time of writing. These documents are revised and superceded from time to time. The most up to date version should always be used for reference.