PROFESSIONAL FOOD MICROBIOLOGY GROUP (PFMG)

ACCREDITATION ADVISORY GROUP GUIDELINES

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Professional Food Microbiology Group (PFMG)
Accreditation Advisory Group Guidelines

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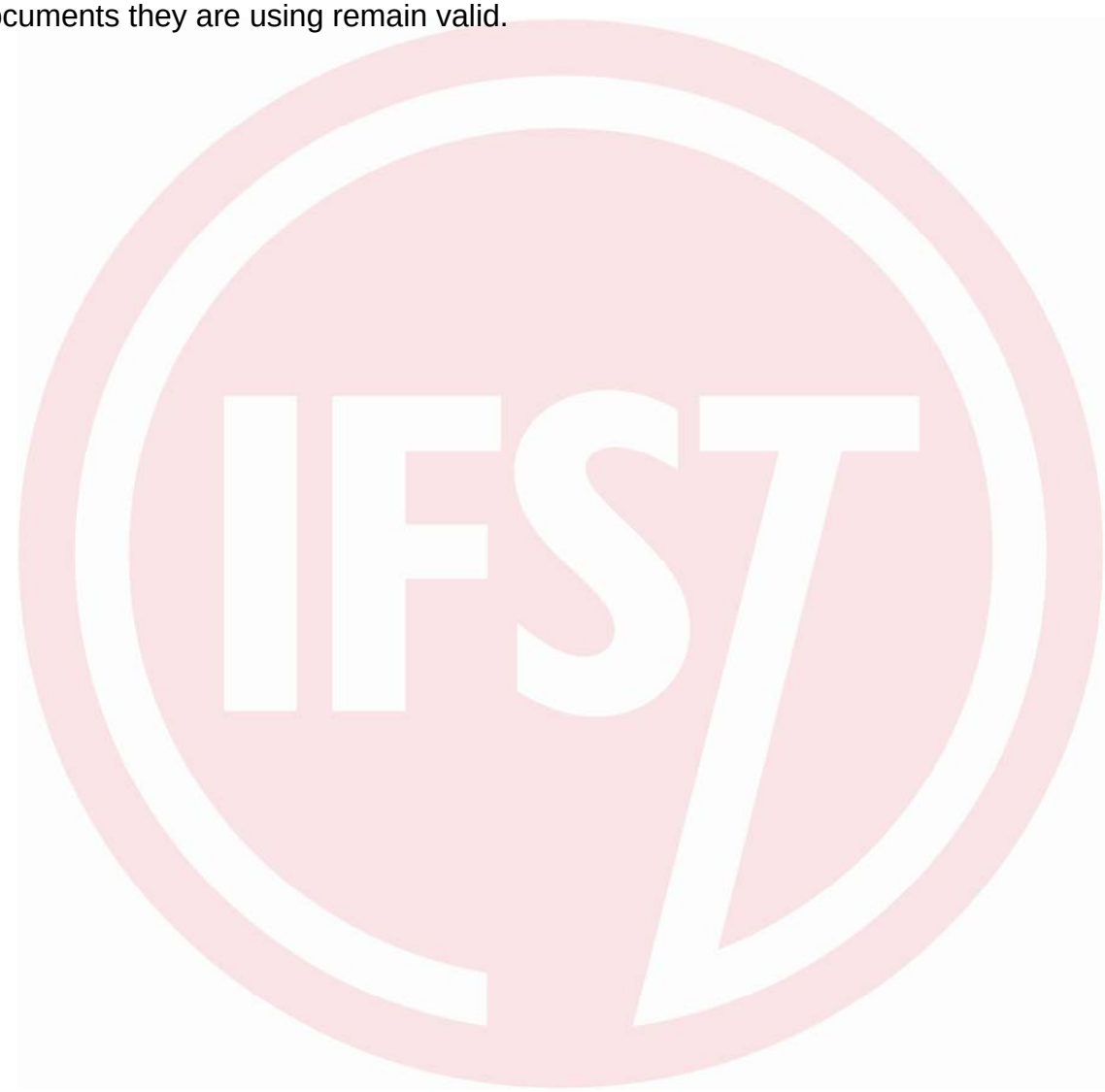
General Information

British Standards and International Standards are reviewed periodically and may be confirmed, amended, withdrawn and / or superseded. Users of these documents should regularly check that the Standard references they are using remain valid.

The publications of the United Kingdom Accreditation Service and associated organisations are reviewed periodically and users should regularly check that the publications they are using from these organisations remain valid.

These PFMG Accreditation Advisory Group Guidelines are also reviewed periodically and may be updated or revised. Users of these Guidelines should regularly check that documents they are using remain valid.



GUIDELINE NO. 1, 12/95, confirmed 12/98:
RECOMMENDED CHECKS FOR MECHANICALLY DRIVEN SPIRAL PLATERS

General

This guideline document should be used in addition to the manufacturer's instructions. It is important to regularly check that a spiral plater is set-up correctly if accurate results are to be obtained. The spiral plater must be kept clean and regularly serviced.

The quality of the agar plate used on the spiral plater is vital to the accuracy of the system.

Agar in plates should be of regular depth, flat and even without any bubbles or blemishes. There must not be any excess moisture on the surface of the agar; but, over-drying should also be avoided.

For correct calculation of results it is essential to:-

- know the volume dispensed by the plater. This is fixed according to the model of plater being used;
- use the correct tables for each spiral plater

Calibration of Spiral Platers

The Spiral Plater can be calibrated by the supplier / manufacturer or other suitably qualified person. This should be done at least annually.

Re-calibration may need to be carried out following service or repairs, or failure of calibration checks (see below).

Checking of Spiral Platers

a) Each day before commencing sample processing, plate a control diluent to ensure that the proper volume will be dispensed. The following checks can be made:-

i) The cam follower arm rests on the cam at the correct height.
ii) The Petri dishes to be used fit the turntable and that they are centred correctly.
iii) The starting and finishing points of the stylus tip on the agar plate are correctly aligned on the plate according to manufacturer's instructions and that there are no gaps in the dispensed spiral.
iv) The stylus tip automatically lifts from the agar surface at the proper time.
v) The state of the Teflon tip of the stylus is satisfactory e.g. free of physical damage, blockage.

If necessary, the relevant adjustments should be made as described in the Spiral Plater Manual.

During plating, check that the system fills with test sample inoculum without any air bubbles and that no bubbles interrupt the volume being dispensed onto the plate.

b) Monthly checks:-

i) The angle at which the stylus tip touches the agar. This is the left-right angle and the heel-toe angle determined with the help of the plastic levelling aid and the level gauge described in the manual.
ii) Dye test to ensure that the sample is deposited evenly without any "skips".

c) It is recommended that checks to confirm the calibration of each machine are carried out at least twice a month, irrespective of external calibrations employed. However, the frequency with which any checking is carried out will be dependant on usage.

Examples of how this may be done are:-
i) dispense spirals onto a filter paper and check gravimetrically. This requires a calibrated, highly accurate analytical balance, or
ii) assess in parallel against a standard plate count method.

d) Bacterial calibration is also available from the manufacturer of the Spiral Plater (details available from the PFMG Accreditation Advisory Group).

Tolerances

Tolerances set should be clearly specified.

An example of tolerance set for calibration checks using conventional plating in parallel is ± 0.5 log cycle applied to the results (Jarvis *et al*., 1977).

The tolerance **must not be more** stringent than the intra-laboratory measurement of uncertainty (see Guideline No. 13 in this series, ‘Uncertainty of Measurement in Food Microbiology’ by Analysis of Variance.

Servicing

Spiral platers must be maintained in good working order and serviced regularly by a suitably qualified person e.g. annually.

Documentation

Procedures relating to calibration, servicing and checking of spiral platers should be fully documented and all relevant data recorded.
Spiral platers must be clearly marked detailing the last and next dates of service and calibration.

Reference:

Jarvis *et al.* (1977) Evaluation of the Spiral Plate Maker for the Enumeration of Microorganisms in Food. *Journal of Applied Bacteriology* **43**, 149-157.

GUIDELINE NO. 1A, 12/98, updated 12/03
RECOMMENDED CHECKS FOR AUTOMATIC SPIRAL PLATERS

Introduction

This guideline document should be used in addition to the manufacturer's instructions. It is important to regularly check that a spiral plater is set-up correctly if accurate results are to be obtained. The spiral plater must be kept clean and regularly serviced.

The quality of the agar plate used on the spiral plater is important with respect to the accuracy of the system.

Agar in plates should be of regular depth, flat and even without any bubbles or blemishes. There must not be any excess moisture on the surface of the agar; but, over-drying should also be avoided.

For correct calculation of results it is essential to:-

- know the volume dispensed by the plater which is fixed according to the operation mode being used
- use the correct tables for each spiral plater.

Calibration of Spiral Platers

The Automatic Spiral Plater should be set up in accordance with the performance specifications provided by the supplier/manufacturer. This should be done at least annually. This may need to be carried out following service or repairs, or failure of calibration checks (see below).

Checking of Spiral Platers

a) Each day before commencing sample processing, the following checks should be made:-

i) Check that the turntable is completely level.
ii) Check that the petri dishes to be used fit the turntable and are correctly centred.
iii) Initiate the test routine to ensure that the stylus tip start and finish points are correctly set.
iv) Check the condition of the stylus tip e.g. free of physical damage, blockage etc.
v) Draw several millilitres of water through the stylus looking for any evidence of leakages in the system.
vi) Run a dye test. Whilst the system fills with dye, check that no air bubbles are visible in the sight glass. As the dye is plated out check that the sample is deposited evenly without any surface skipping.

b) It is recommended that checks to confirm the performance of each machine are carried out at least twice a month, irrespective of external calibration service used.

However, the frequency with which any checking is carried out will be dependant on usage. Examples of how this may be done are:-

- Dispense spirals into a sealable container, keep the water in a single droplet and avoid warming the container with the hand to avoid evaporation, and check gravimetrically. This requires a calibrated, highly accurate analytical balance, or
- Assess in parallel against a standard plate count method.

c) Bacterial calibration is also available from the manufacturer of the Spiral Plater (details available from the PFMG Accreditation Advisory Group).

Tolerances

Tolerances set should be clearly specified.

An example of tolerance set for calibration checks using conventional plating in parallel is 0.5 log cycle applied to the results (Jarvis *et al*., 1977).

The tolerance **must not be more** stringent than the intra-laboratory laboratory measurement of uncertainty (see Guideline No. 13 in this series, 'Uncertainty of Measurement in Food Microbiology' by Analysis of Variance.

Servicing

Spiral platers must be maintained in good working order and serviced regularly e.g. annually, by a suitably qualified person.

Documentation

Procedures relating to calibration, servicing and checking of spiral platers should be fully documented and all relevant data recorded.

Spiral platers must be clearly marked detailing the last and next dates of service and calibration.

Reference:

Jarvis *et al.* (1977) Evaluation of the Spiral Plate Maker for the Enumeration of Micro-organisms in Food. *Journal of Applied Bacteriology* **43**, 149-157.

GUIDELINE NO. 2, 12/95, revised 6/03: RECOMMENDED CHECKS FOR AUTOCLAVES

This guideline document should be used in conjunction with the appropriate manufacturers' instructions and with reference to the following documents:

EA-04/10, Accreditation for Microbiological Laboratories (July 2002). www.european-accreditation.org.

British Standard BS2646 parts 1-5 (1993) Autoclaves for sterilisation in laboratories. British Standards Institution, London, UK.

Directories of United Kingdom Accreditation Service (UKAS) Accredited Organisations: www.ukas.com

Scope

This guideline is designed to provide guidance to laboratories on the specific requirements for calibration, validation and process monitoring checks for autoclaves and media preparators necessary to comply with good laboratory practice (GLP) and the standards of assessment or Accreditation Bodies.

Definitions

Validation is the provision of evidence using specified procedures that the equipment operating cycles are capable of repeatable effective performance i.e. for autoclaves, checking that the selected operating cycles applied to different load types are capable of achieving the required process conditions in loads representative of those typically used by a laboratory.

Calibration is an accurate determination, based on all process parameters, of continuing compliance to a predetermined target by measurement against acceptable standards i.e. for autoclaves, using recognised reference devices to compare against temperature and time settings in order to verify that targets are being repeatedly achieved.

Process monitoring is an indication, based on one or more parameters, of compliance to the predetermined target i.e. for autoclaves, using a calibrated maximum thermometer or chemical or biological process indicators.

Throughout the guideline, items referred to as a 'must' are essential to comply with specific Accreditation requirements. Items referred to as a 'should' are desirable and infer suitable GLP.

Introduction

The culture properties of the media used in microbiological testing are critical to the quality of test results produced. Since excessive heat treatment of media may result in nutrient destruction, pH drift, darkening, precipitation, poor gel strength and reduced cultural performance, it is important to optimise the heating process so that the medium is sterile after heating, whilst causing minimal damage to its composition.

The general instruction given by media manufacturing companies for the sterilisation of media volumes up to 1 litre is 121°C for 15 minutes (apart from heat sensitive media where lower temperatures and / or shorter process times are required). To ensure that media has not been adversely affected by the heat process applied, both autoclave and media performance must be assessed.

Autoclave Assessment: Load validation

A validation of the suitability of the autoclave / media preparator must be made as part of its initial commissioning into a laboratory. This is referred to as *Load Validation* and should only be performed as a one off exercise unless there is a significant repair or modification to the unit that is likely to alter its process parameters, i.e. replacement of a thermo-regulator probe and controller assembly, change to loading arrangements or change in operating cycle.

The process efficacy of the autoclave should be validated for all types of media and bottle sizes used by the laboratory, however, this is often impractical and time-consuming. It is acceptable to validate different load sizes by placing the temperature probes inside bottles containing tap water, as this will provide comparable heating and cooling profiles to laboratory media. Where possible, autoclave loads should consist of containers of a similar volume. Where this is impractical, suitable validation data must be generated for each different volume within a load showing that each receives a suitable process and are neither over- nor under-processed. This involves placing a temperature probe inside the smallest and largest sized containers and checking the process received.

Load validation must be carried out by:

- an appropriate external agent holding relevant Accreditation i.e. UKAS for calibration and testing or,
- in-house by competent staff using a valid and fully documented procedure which meets UKAS requirements

The validation is performed by placing a series of National Physical Laboratory (NPL) traceable thermocouples throughout loads representative of the different types of load expected to be processed in the laboratory e.g. waste, media and equipment, and running the autoclave through the desired cycle. All data is collated from the thermocouples and maximum temperature differences between different points throughout the chamber are calculated. The difference is then used to determine the

uncertainty of measurement value when applying a workable tolerance to the temperature performance of the autoclave.

The number of thermocouples used will vary depending on the vessel size, but should be sufficient to adequately evaluate the effects of location and loading on the temperature achieved. Further guidance is given in the British Standard (BS) 2646 Part 5.

For Media Preparators, process validation should demonstrate uniform heating throughout the vessel. At least two temperature sensors should be used, where one is placed adjacent to the control probe and the other is immersed in the medium throughout processing.

Calibration

Calibration of autoclaves must be performed at least annually. This frequency may be increased if unreliable equipment is found to drift from set values or after any repairs, which involve adjustment / replacement of the temperature controlling devices.

Calibration can be performed either by:

a) A UKAS accredited external agent (see UKAS *Directories of Accredited Organisations*) or

b) In-house using competent staff following a suitably documented procedure

The following points must be noted:

All temperature controlling and monitoring devices and the process timer must be checked against NPL traceable references. The calibration should cover all temperatures used by the laboratory.

All data obtained must be recorded, collated and evaluated to check that the chamber achieves the set temperature within the required tolerance previously determined under validation. Laboratories must specify the minimum temperature and time conditions required to ensure that adequate sterilisation is achieved for each load type. Any adjustments necessary to ensure complete alignment between the traceable reference, and the test equipment read-outs should be made or, any deviations must be noted and accounted for when routinely using the equipment (it is preferable to have alignment rather than correction factors).

The results of calibrations must be clearly documented and must include:

- identification of the autoclave under test,
- identification of the traceable reference devices used,
- an indication of the autoclave and reference thermocouple(s) position(s) within the
- chamber,
- the operating parameters used, i.e. temperature(s) and time(s)

- the readings obtained from all monitoring devices both before and after correction,
- all raw data generated including any chart printouts.

External agents providing the calibration service should normally supply this information on the certificate of calibration for each item of test equipment (autoclave or medium preparator).

Notes:

1) Load validations and calibrations of autoclaves and media preparators are important, but are costly exercises. It is therefore important to ensure that appropriate work is done each time. Laboratory staff must ensure that they understand the results of calibrations and load validation studies generated by either the subcontractor or themselves, and react to these results accordingly.

2) It is permissible for external calibration agents to either, check reference thermocouples on site using a controllable and reliable heat source e.g. boiling water or an oil bath, or to use pre-calibrated probes.

3) Autoclave temperature sensors and read-outs can be calibrated either as a combined unit against a reference sensor or separately by electrical input from a calibrated source to the read-out.

4) For in-house calibrations the autoclave or media preparator timer can be checked against a calibrated timer or against the speaking clock, and must be calibrated at the same time as the temperature controller / sensor is calibrated.

5) The most common limiting factor to performing an effective in-house calibration is the absence of a controllable and reliable heat source that will provide an accurate and well-maintained temperature.

6) In-house calibration methods must be fully documented and verified by the laboratory's assessment / accreditation body.

Process Monitoring

Process monitoring must be performed for every autoclave run. A variety of methods are available and the most reliable are chamber or load probes linked to digital or chart recorders. Results obtained from the process monitor must be checked for each run to ensure that an adequate process has been achieved.

Alternatively a maximum thermometer may be used, but this will not provide information on temperature fluctuations throughout the process or an indication of the time achieved at the required temperature. Other process monitors such as chemical and biological indicators e.g. Brownes Tubes, Thermologs, spore strips, can be used and are accepted as process indicators by assessment and accreditation bodies where probe / chart

equipment is not available. Also, these are commonly used to support chart records where these are used.

Autoclave tape must not be used as a monitor of the autoclave process, but simply as a visual indicator of whether a load has been autoclaved or not.

For autoclaves and media preparators clear acceptance and rejection criteria must be set for process monitor checks and records maintained.

Process control

Autoclaves are fitted with a process control probe that activates the sterilisation timer when the required internal temperature is reached.

For media processing, the chamber probe controlling the process can be left in the open chamber or alternatively can be placed into a bottle of water. It is recommended that for volumes of 100ml or greater, the probe is placed into a bottle of water representative of the load.

For waste processing the controlling probe should be located in a holder inside the autoclave chamber or on the load support. Over-processing is not an issue with waste.

For media preparators the controlling probe is always an in-load probe. In this case this is acceptable since over-processing is unlikely as the media is continually stirred therefore giving an effective heat distribution and allowing accurate temperature control for the entire load.

Note: There is much debate over the positioning of the temperature probe during media sterilisation cycles. The standard processing parameters for many media are 121°C for 15 minutes for 1 litre volumes and traditional steam pressure vessels used in laboratories were always chamber temperature and pressure controlled and operated. Today, there are many more complex electronically controlled autoclaves operated by load temperature probes, but media manufacturers' processing instructions have remained the same i.e. they do not specify whether the target heat process should be achieved in the chamber of the autoclave or within the media.

An advantage of using a load probe is that the actual temperature achieved in the media is measured, however, the media receives much more heat in this system with the risk of over-processing which is particularly important in relation to heat-sensitive media. The benefit of a chamber probe is that the temperature within the autoclave chamber is monitored and as soon as the temperature / time process has been achieved the autoclave will start to cool down. The disadvantage of this system is that the exact process received by the media cannot be determined and it is possible that larger volumes of media are under-processed. Whichever process type is used, it is essential to understand, through process validation, how the heat is applied to and affects, the

media. To support this requirement, media performance monitoring is essential to demonstrate the efficacy of each batch produced.

Verification of Autoclave Process by Media Performance Assessment

Verification of autoclave process efficacy needs to be carried out routinely for media prepared and processed in the laboratory. Such verification is achieved via a robust system of media quality control (QC) checks that must include a check of pH, appearance, media performance (by inoculation with appropriate microorganisms that demonstrate the productivity, selectivity etc. of the medium) and sterility.

For media efficacy, inoculation methods may be semi-quantitative by comparison of growth zones from a streak plate on the test medium to zones on a non-selective medium or, may be quantitative, by comparing growth productivity compared to a 'standardised batch'. Some media manufacturers' Manuals give guidance on these methods.

For media performance, clear acceptance / rejection criteria must be set and records maintained.

Servicing of Autoclaves

Autoclaves must be maintained and serviced regularly. Such operations must be in accordance with the manufacturer's instructions and BS 2646 part 4.

The frequency of servicing and maintenance should take account of the manufacturer's recommendations, the reliability and stability of the unit, the extent of use and possibly other factors such as water quality which will determine the level of deposits (scale) within the steam generator.

Note: During servicing, if replacement of essential process controlling units is required, a re-calibration must be performed.

General Considerations

Autoclaves and media preparators for use in microbiology laboratories must be equipped with a minimum of a temperature gauge, pressure gauge, integral process timer and a safety valve. They must be capable of attaining a temperature of at least 121°C and holding times of 45 minutes (alternative equivalent processing must be allowed for, e.g. 126°C for 30 minutes is sometimes used for waste processing).

An initial steam purge must be performed prior to processing in order to remove any pockets of air trapped within a load.

A thermal lock must operate to prevent opening until the load temperature is below 80°C. In some autoclaves this is achieved by use of a probe located within a load simulator that

controls the temperature lock. Care must be taken when using these during media processing as over-processing can be caused due to the increase in the load holding time at the end of the process.

When loading an autoclave for media processing, similar volume and shape of vessels must be used. For example a load should not be made up of 10ml and 500ml containers. This is to minimise differences in heat process received arising due to differences in heat penetration in the different container volumes. The standard processing conditions of 121°C for 15 minutes advised by media manufacturers refer to 1 litre media volumes although in GLP guidelines, maximum container / volumes of 500ml of media are generally recommended to be autoclaved. For large volumes e.g. 2 - 5 litres, a media preparator can be used. In all cases, verification of process effectiveness will be supported via media QC as outlined above.

Where possible, it is advisable to avoid autoclaving very small volumes of media e.g. 10ml because of the relatively high proportion of evaporation and potential for over processing. Such small volumes of sterile media are best obtained by aseptically dispensing a larger volume of sterilised medium to the required size and number of sterile containers. This should ideally be done in a laminar air-flow cabinet to reduce the risk of contamination and the occurrence of false positive results. Alternatively, it is possible to establish 'over-fill' volumes of media prior to autoclaving that take into account losses caused by evaporation during processing and thus producing the correct volume after the autoclave process. Post-autoclave volume checks must be carried out in order to ensure that the correct volume has been achieved and records of these checks must be held.

Media containers must be placed within the autoclave without over-crowding in order to allow adequate steam penetration of the load and prevent air pockets from forming during steam purging.

If possible, rapid cooling of the autoclave post processing should be applied and the media removed from the autoclave as soon as safely possible to prevent over-processing.

For waste loads, care must be taken not to overload the chamber. If bagged waste is packed in too tightly, effective steam penetration will be inhibited resulting in inadequate heat processing and unsafe waste. Ideally, the manufacturer's waste container(s) should be used as they are designed to allow maximum steam flow.

Further information

1. Equivalent heat processes

   It is possible to use lower temperatures with longer processing times or higher temperatures and short times to achieve a heat process equivalent to the standard 121°C for 15 minutes used for many media. This requires the

determination of the heat profile of each autoclave in the laboratory and application of equivalence data to determine an appropriate equivalent process. However, such studies are not easy to perform and do not allow readily for the difference between processes commenced in a cold and pre-warmed autoclave. It is generally considered that the most effective kill is achieved by using higher temperatures for short times rather than lower temperatures for longer times. Details on use of equivalence data can be obtained from Campden & Chorleywood Food Research Association, Chipping Campden, UK.

2. Application of F_o values to media heat processes

The application of F_o values has been suggested as a means of ensuring that optimal heat processing conditions are applied to media and this approach may be used in the future by media manufacturers.

Each F_o unit essentially represents a heat process equivalent to one minute at 121°C. Suitable ranges of F_o values to provide adequate sterilisation without over-processing would need to be established for each medium, ideally by the media manufacturers.
For example, if an F_o range of 4 to 20 was to be recommended for a specific medium this would mean that an equivalent heat process of between 4 and 20 minutes at 121°C is required to achieve sterility. Thus, a minimum holding time of 4 minutes at 121°C (or an equivalent process) would be sufficient to achieve a sterile medium and medium performance would not be affected if times up to 20 minutes at 121°C (or an equivalent process) were used. Processes of 121°C for times in excess of 20 minutes (or an equivalent process) would adversely affect the performance of the medium. In practise a laboratory would decide on a suitable processing time within the recommended range, e.g. F_o of 12 minutes could be applied and the process would be monitored using a load probe.

The advantage of this approach is that media would receive an optimum process with a sensible tolerance and therefore deliver optimum performance.

GUIDELINE NO.3, 12/95, updated 12/03: RECOMMENDED CHECKS FOR BALANCES

Introduction

This guideline document should be used in conjunction with the manufacturer's instructions and with reference to the following documents:-

EA-04/10, Accreditation for Microbiological Laboratories (July 2002). www.european-accreditation.org
LAB 14 Calibration of Weighing Machines. United Kingdom Accreditation Service, Feltham, UK.

It is important to ensure that a balance is sufficiently accurate for intended uses, that it is fully calibrated and that the calibration status is regularly checked. The balance must be kept clean and regularly serviced.

Balances must be sited in a level, draught and vibration-free position so that steady readings can be obtained. They should not be subjected to rapid environmental change or sources of dust.

Calibration of Balances

An annual calibration of each balance covering the range of use is required. This may be performed by a UKAS accredited calibration agent. Alternatively, balances may be calibrated in-house using the procedures outlined in LAB 14.

Re-calibration may need to be carried out following service or repairs, or failure of daily checks (see below).

Weights used for calibration purposes must be:

i) Used only for calibration purposes.
ii) Capable of providing the necessary accuracy as documented in LAB 14.
iii) Calibrated to National Physical Laboratory (NPL) traceable standards.
iv) Re-calibrated at defined intervals e.g. every 2 years.

Checking of Balances

Before each use, check that:-

a) the balance is clean, level, zeroed and in calibration.

b) Daily when in use, or after cleaning, perform the following checks:-
Zero the balance. Using clean cotton gloves, tweezers or cloths, place a check weight (or stable mass of known weight) which falls within the range of use of the

balance, on the centre of the pan. Record the reading obtained, this should be within the tolerance as specified. Remove the weight, the balance reading should return to zero. If there is failure to meet the appropriate criteria, the laboratory's 'out of specification procedures' should be followed.

When conducting balance checks, a weight appropriate to the range of use of the balance must be used. Only one weight is required for the check, however it is good practice to vary the weight.

c) Further checks:-
Optional intermediate checks on linearity using a range of weights may be performed between calibrations.

It is good practice to periodically conduct linearity checks using a range of suitable weights. The frequency of such checks depends on the use of the balance.

For daily checks of a balance, weights used can either be:

NPL traceable certified weights,
OR
Weights of a known mass as determined in-house. The actual mass can be determined on a balance immediately following calibration.

Tolerances

Tolerances set should be dependant on application of use and must be clearly specified.

Examples of tolerances could include:

a) Readings (including zero checks) should not deviate from the expected by more than one digit in the final decimal place.
b) Specify a percentage or weight deviation from the target weight e.g. 10g ± 5% or 10g ± 0.1g.

Servicing

Balances must be maintained in good working order and serviced regularly, eg annually, by a suitably qualified person.

Documentation

Procedures relating to calibration, servicing and checking of balances should be fully documented and all relevant data recorded.

Each balance must be clearly marked detailing the last and next dates of service and calibration.

GUIDELINE NO. 4, 12/95, updated 12/03: QUALITY ASSURANCE OF LABORATORY CONSUMABLES

Introduction

Quality is concerned with reliability and prevention of errors and cannot be applied as a veneer by inspections and audit procedures aimed solely at correcting errors. The aim of this guideline is to advise laboratories on both Quality Assurance (QA) and Quality Control (QC) information that should be obtained from suppliers of laboratory consumables. It is concerned with establishing the accreditation status of the supplier and the ability to supply performance data relating to their products.

Establishing the QA system and registration status of the supplier

The laboratory should write to the supplier and ask for details of their QA system and establish the registration / certification status e.g. British Standard BS EN ISO 9001-2000. A copy of the registration certificate and details of the scope of registration should be obtained. This certification should cover product manufacture, supply and distribution.

Product Quality Specification

The manufacturer should initially supply a dated and signed Quality Specification which should include at least the following information where applicable:

a) Company name
b) Product description / formulation
c) Shelf life of the product
d) Normal batch size
e) Storage conditions required, including temperature
f) Method of preparation
g) Sampling regime/rate
h) Sterility checks including acceptability criteria
i) Volumetric and gravimetric data
j) Efficacy checks including the organisms used, their culture collection reference and the acceptability criteria
k) Date of issue of the specification

When this information changes, the manufacturer should supply a revised specification.

QC by the supplier and performance data on certificates

Each supplied batch / lot should include an "Assurance Certificate" which indicates that the batch in question has passed the specifications as laid down in the Quality Specification.

Laboratory suitability and in-house QC

The laboratory must initially verify the suitability of the product for the purpose intended. Further checks on the product must be made on a random basis to ensure continued compliance with the product's specifications. These checks should be included in the laboratory's in-house QC programme.

Challenge of suppliers

It is essential that laboratories check that suppliers are holding full QC data on batches of products. Suppliers should be occasionally challenged to produce this information for batches of consumables received by the laboratory. It is advisable to include this in an annual audit schedule to ensure that such checks are conducted regularly.

References and further reading

British Standard BS 4778-3.1:1991 Quality Vocabulary. Availability, reliability and maintainability terms. Guide to concepts and related definitions.

British Standard BS EN ISO 9001:1994. Quality systems. Model for quality assurance in design, development, production, installation and servicing.

British Standard BS EN ISO 9001:2000. Quality systems. Requirements.

British Standards are available from the British Standards Institution, Customer Services, 389 Chiswick High Road, London W4 4AL, UK

GUIDELINE NO. 5, 4/97, confirmed 6/00: MEDIA STORAGE AND PREPARATION

Introduction

This guideline document should be used in conjunction with manufacturers instructions and with reference to the following documents:

1. Campden & Chorleywood Food Research Association (CCFRA) Guideline No. 9 (1996): *A Code of Practice for Microbiology Laboratories Handling Food Samples (incorporating CCFRA Technical Manual No 33: Guidelines for the Storage, Preparation and Handling of Microbiological Media).* CCFRA, Chipping Campden, UK.

2. Professional Food Microbiology Group AAG Guideline No. 2: Recommended checks for autoclaves.

3. Professional Food Microbiology Group AAG Guideline No. 3: Recommended checks for balances.

4. Professional Food Microbiology Group AAG Guideline No. 4: Quality Assurance of Laboratory Consumables.

5. Professional Food Microbiology Group AAG Guideline No. 9: Part I. Recommended checks for pH meters and Part II Measuring Media pH values.

General

This Guideline provides guidance on those points which require careful attention when handling, preparing and storing microbiological culture media, to ensure that media performance is not impaired, thereby assisting the production of consistently accurate and reliable microbiology test results.

The Guideline is split into 3 sections for ease of reference:-

- Dehydrated Media and all Supplements
- Pre-prepared Media
- Media prepared by the user

Health And Safety

Care must be taken when handling all media and supplements. The manufacturer's instructions must be followed and staff should be fully aware of relevant hazard data information supplied.

DEHYDRATED MEDIA AND ALL SUPPLEMENTS

Receipt

HAZARD - Contaminated Media.

CONTROLS

- Only approved suppliers should be used and media should be bought to an agreed specification.
- Media should be ordered in quantities appropriate to the rate of use in the laboratory.
- The availability of the manufacturer's QC results should be ensured, if they should ever be required.
- Condition of deliveries should be checked on arrival e.g. intact seals, undamaged containers, and should be rejected and returned to the supplier if found not to be satisfactory.

Media Storage

HAZARD - Deterioration of media quality.

CONTROLS

- Media should be stored in accordance with manufacturer's instructions e.g. in dry and cool conditions, away from bright light, refrigerated.
- Storage of dehydrated media in areas of high humidity should be avoided e.g. autoclave rooms or refrigerators, as this can cause condensation in the airspace of storage containers.
- Good stock control should be observed. This can be assisted by labelling with date of receipt, date of opening and expiry date. The lot number and the first-use date should be recorded.
- The shelf life of unopened dehydrated media is commonly 3 - 5 years, but this should be checked with the manufacturer.
- The shelf life of media is reduced once opened, and should be established by the laboratory by visually checking that it remains free flowing, dry and free from lumps.
- The ‘use-by’ date of containers should always be checked before use and closures should be replaced tightly immediately after use. Any out-of-date media and supplements should be discarded.

Water Quality

HAZARD - Use of sub-standard quality water affecting the quality of the final reconstituted media.

CONTROLS

- Fresh distilled, de-ionised or reverse osmosis water should be used.
- Tap water or distilled water from boiler condensate should not be used as they may contain impurities which can concentrate in the media and inhibit growth of microorganisms.
- All equipment used for preparation of de-ionised and distilled water should be properly maintained and serviced.
- Water should ideally be used on the day it is produced. If water must be kept, it should be stored for as short a time as possible in an inert, covered container. Glass containers are best, and use of the following containers should be avoided: soda glass (causes alkali drift), polythene (causes acid drift) and metal (metal ions leached into water).
- The conductivity of water should be checked and recorded at least daily, or before each use if water is not used daily. A suitable conductivity meter should be used, that is regularly calibrated against a standard solution of known conductivity. A conductivity tolerance must be stipulated. This should be a low reading e.g. less than or equal to 10 micro Siemens (μS). If the conductivity of water exceeds the specified tolerance, then the laboratory must investigate the reason for this. In such circumstances the water should only be used if the laboratory can demonstrate that the performance of media will not be adversely affected.

Reconstitution of Media

HAZARDS

- Use of sub-standard dehydrated media.
- Use of sub-standard water (see previous section).
- Inaccurate weighing out / dilution.
- Cross contamination.
- Inadequate mixing / dissolving in pre-heating process.

CONTROLS

- Media should be checked to ensure that it is within date / shelf life, and if not it should be discarded.
- Media should be checked for visible signs of deterioration e.g. discoloration, loss of flow, increased granulation / formation of lumps. Media should be discarded if there are signs of deterioration.
- The correct amount of powder should be weighed out using a clean, dry spatula. Direct pouring from the container should be avoided as this creates dust and can lead to inaccurate weighing. Inhalation of irritant dusts should be avoided, and may be prevented by the use of face masks or dust hoods.
- A calibrated balance (accurate to 0.01g) should be used and, for weighing powders, should be read to 1 decimal place.

- The required amount of water should be obtained using a measuring cylinder. Alternatively, water may be added by weighing out into a tared vessel.
- Good quality glassware should be used i.e. not chipped or scratched, which has been adequately washed and rinsed in de-ionised water. The glassware used should also be of a sufficient size to allow good mixing e.g. twice the volume of the media.

- To ensure good dispersion of the media in the water, the following protocol is recommended prior to sterilisation :-

  1) Mix well with the water.
  2) Allow to soak for 15 minutes.
  3) Mix again.

Sterilisation - Boiling, Autoclaving, Use of Agar Preparators

HAZARDS

- Insufficient heat treatment causing non-sterile media.
- Too harsh a treatment causing denatured media.
- Cross contamination via any tubing used to dispense media.
- Confusion between sterile and non-sterile media.

CONTROLS

- Autoclaves (see: PFMG AAG, Guideline 2, Recommended checks for autoclaves)
- Autoclaves must be regularly serviced and calibrated (to include process temperature and time).
- Media should be loaded into the chamber so as to allow adequate steam circulation around the load.
- Similar volumes should be processed together.
- Ideally, individual volumes greater than 1 litre should not be autoclaved. If such volumes are necessary, then process validation information must be obtained.
- Media manufacturers' recommendations should be followed for autoclave processing. These are typically 121^{o}C / 15 psi / 15 min. However, some media will require processing at lower temperatures.
- Each autoclave load should be monitored using a calibrated thermocouple and chart recorder, or a calibrated maximum thermometer. In addition, spore strips, Brownes tubes or thermolog strips may be used (autoclave tape must not be used as a process monitor).
- Autoclave tape should be attached to each load to enable processed and non-processed media to be distinguished apart.

CONTROLS - Media Preparators

- Manufacturer's instructions should be followed carefully.
- Equipment should be regularly cleaned, maintained, serviced and calibrated.
- Tubing must be cleaned and sterilised after each use. There should be dedicated, separate tubing for each different medium used.

CONTROLS

- Boiling e.g. XLD agar and VRBA agar
- Good mixing should be ensured during boiling to prevent localised hot spots developing, e.g. by use of a hot plate with a magnetic stirrer.
- These media should not be allowed to solidify and then be re-melted and used.

Storage of Rehydrated / Sterilised Media

HAZARDS

- Deterioration of media resulting from photo and chemical oxidation and dehydration.
- Contamination of media during storage

CONTROLS

- Media containers should be marked clearly with the medium's identity, the date of preparation and / or the use-by date. Good stock rotation controls should be in place.
- Media should be stored according to manufacturer's instructions e.g. away from light, at the correct temperature (2-8^{o}C or room temperature), in plastic bags if required, with caps of containers tightened. Some media are not suitable for storage, but this will be indicated in the manufacturer's instructions.
- Plates should be stored medium side up i.e. lids on the bottom. Laboratories should establish an appropriate shelf life for each type of prepared medium under appropriate storage conditions. This should include a demonstration that microorganism recovery and performance are not adversely affected. Typical storage times are 5 days at 2-8^{o}C for agar plates not in bags, and 7 days for those in bags.
- Non-selective bottled media may typically be kept for 4 months at 2-8^{o}C. Bottled media stored at room temperature has a shorter life which should be determined by shelf life testing.
- Media should be visually checked prior to use for signs of deterioration or contamination e.g. colour, microbial growth, precipitation.
- Positive and negative microbial growth controls should be carried out on the media. This may be done prior to use or alternatively may be done in parallel with use of a medium e.g. VRBA (see section on Media QC). Certain physical checks

i.e. pH value and volume, should also be done at this stage (see section on Media QC).
- If media is found to be unsatisfactory, or QC check results are out of specification, then it should be discarded or other appropriate action taken.

Re-melting of Media - e.g. Free steaming in an autoclave or using a boiling water bath

HAZARDS
- Contamination due to water or ingress of steam.
- Denaturation due to localised hotspots.
- Prolonged exposure to high temperatures once melted

CONTROLS

- The minimum times required to re-melt media should be established for the different volumes used in the laboratory. Results should be documented and adhered to.
- Prior to re-melting, media should be checked for any visible signs of microbial contamination, and discarded if necessary.
- The use of direct heat to re-melt media should be avoided e.g. bunsen burner, hot plate / ring without heat diffuser.
- Autoclaves with free-steaming capability may be used.

Use of boiling water bath:

- The water level in the bath should be maintained above the level of the medium but below the neck of the bottle.
- To minimise the risk of overheating, media containers should always be placed directly into the boiling water.
- Bottles should be appropriately balanced in the bath to prevent them falling over.

Media Tempering

HAZARDS
- Heat denaturation leading to agar softening and loss of growth / selective properties.
- Contamination via ingress of water.

CONTROLS

- After re-melting, media should be allowed to cool at room temperature for 5-10 minutes before being transferred to a tempering bath. This will prevent thermal shock and an increase in water bath temperature.

- Media should usually be tempered to 46^{o}C +/- 1^{o}C before use. However, some media which require the addition of supplements e.g. Baird Parker and PREP, should be tempered to 50^{o}C in order to prevent the medium from solidifying during the addition process.
- The water level in the bath should be maintained above the level of the medium but below the neck of the bottle.
- The waterbath should not be overloaded. Adequate space for water circulation should be allowed.
- Use of waterbaths with lids should be avoided, as this may cause contamination via condensation. Plastic floating balls may be used to help prevent excessive evaporation.
- Water in the baths should be changed regularly. Waterbaths should be cleaned regularly. They may also be sterilised e.g. 80^{o}C/15-30 minutes, or treated with a biocide if necessary.
- Sufficient time should be allowed for the media to cool to the required temperature before it is used. Trials may need to be carried out to determine such times and a documented tempering procedure should be in place. Media should be used as soon as possible after equilibration to the required temperature.
- In general, media should not be stored for longer than 4 hours (or 1 hour for acidic media (pH value <5.0). Manufacturer's instructions should be referred to for guidance.
- Media should be checked for any visible signs of deterioration prior to use e.g. colour change, precipitation, poor gel strength.

Supplement Addition

HAZARDS

- Cross-contamination during addition.
- Denaturation of the supplement and / or medium.
- Incorrect preparation e.g. incorrect volume of liquid or weight of medium.

CONTROLS

- When preparing, handling and adding supplements to media, manufacturer's instructions should be followed carefully. Additional care must be taken with media and supplements which are known to be particularly hazardous (refer to manufacturer’s specific product data information).
- When preparing, handling and adding supplements to media, aseptic techniques must always be used.
- A full QC check should be carried out on supplements made in-house from basic components.
- The sterility of supplements must be ensured. Commercially prepared supplements are usually supplied sterile; in-house prepared supplements should be sterilised by an appropriate method e.g. by membrane filtration. As heat can

destroy antibiotic activity, it is essential that manufacturer's guidelines are followed to ensure that the appropriate methods of sterilisation are used.
- The supplement should be dissolved fully (for solids) or thoroughly, but gently mixed (for liquids) prior to addition to media. This will help ensure that the correct concentration in the medium is achieved, and avoid the formation of air bubbles.
- Large volumes of chilled supplements e.g. egg yolk, should be warmed to room temperature prior to addition to the basal medium.
- Supplements should be added to pre-tempered, sterile medium immediately prior to use, and complete mixing should be ensured.

Use of Media - Plate Pouring

HAZARDS

- Cross-contamination.
- Dehydration of media due to incorrectly poured plate.
- Condensation resulting from agar being too hot.
- Heat inactivation of heat labile supplements or microorganisms.

CONTROLS

- To prevent heat inactivation of microorganisms and / or excessive condensation within plates, properly tempered media should always be used.
- The agar container should be carefully removed from the water bath and dried to prevent water running down the side of the container and into agar during pouring.
- Media containers should be swirled gently prior to pouring to ensure an even dispersion of the agar.
- Good aseptic technique should always be used.
- For standard 90 mm Petri dishes (plates), a volume of *c*.15 ml/plate should be use to minimise dehydration.

When using an automatic plate pourer:
- Manufacturer's instructions should always be followed.
- Regular equipment maintenance, servicing and calibration schedules should be in place, and fully documented.
- All tubing should be sterilised prior to use and rinsed immediately after use.
- The condition of tubing should be checked regularly and replaced if splitting or perishing is evident.

- Dedicated tubing should be used for selective media to prevent cross contamination of inhibitory substances.

Drying of agar in Petri dishes

HAZARDS

- Over-drying of plates, resulting in concentration of media nutrients and reduction in water activity which may impair growth.
- Insufficient drying of plates resulting in confluent growth of the inoculum on the agar surface.
- Cross-contamination.

CONTROLS

- Temperature and times of drying should be established for each medium used. These should be documented.
- The maximum temperature for drying should not be greater than 55^{o}C.

If using an incubator or drying cabinet:

- In order to minimise cross- contamination, a specific incubator or drying cabinet should be reserved only for the drying of plates where possible.
- Media should be protected from light.
- Fan assisted equipment should be avoided to prevent over-drying of the media.

If using a laminar air-flow cabinet:

- The cabinet should be regularly disinfected, following manufacturer's instructions.
- A sterile flow of air should be established by running equipment for at least 10 minutes prior to use.
- The cabinet should be loaded with Petri dishes from the back to the front, and collected from the front to the back.
- Lights should not be used in the cabinets.

QC of Media

HAZARD - Poor quality media causing incorrect performance and possible invalid results (see also Appendix 1 of this Guideline).

CONTROLS

- The pH value of the cooled media should be checked against the manufacturer's specification. (see: PFMG AAG, Guideline No.9 (Part II), Measuring media pH values).
- The visual appearance of media should be checked.

- Positive and negative microbial growth controls should be carried out for each batch of medium i.e. positive controls including growth or the correct colony morphology of the target organism on selective media, and negative controls i.e. sterility or correct morphology of non-target organisms.
- Critical volume checks should be carried out on media after autoclaving e.g. on 9ml volumes of diluent.
- A comparative test of old and new batches of media is a useful way to ensure that consistency is maintained between batches.

Records of Media Preparation

Records should be held for all batches of media, both prepared and used, and should include dehydrated media batches used with use-by dates, autoclave processing (temperature, time and pressure monitors), temperature checks of waterbaths, refrigerators etc., and all QC checks. It is essential to be able to demonstrate traceability of media used in any test back through the process to the media batch purchased.

PRE-PREPARED MEDIA

HAZARD - Poor storage conditions causing incorrect media performance and invalid results.

CONTROLS

- Manufacturer's storage instructions should be followed carefully.
- Media should be ordered in quantities appropriate to the rate of use in the laboratory.
- The availability of the manufacturer’s QC results should be ensured if they should ever be required.
- Good stock rotation controls should be in place, as shelf lives are much shorter than for dehydrated media.
- QC checks should be carried out (see PFMG AAG Guideline No. 4: Quality assurance of laboratory consumables), and should include regular media efficacy checks e.g. positive and negative microorganism controls.

MEDIA PREPARED FROM INDIVIDUAL COMPONENTS BY THE USER

HAZARD - Poor preparation leading to incorrect performance of media, and invalid results.

CONTROLS

- All components should be identified clearly and accurately in a list, and records kept of their weight of addition.

- The use of the correct nature and grade of chemicals and agar extracts required should be ensured.

For weighing out of ingredients:

- A clean, dry implement should be used for each constituent. These should not be kept stored in the containers.
- Ingredients should be weighed carefully and accurately into separate containers, e.g. weighing boats, prior to being added together for the final medium.
- Powder should not be taken away from weighed amounts and put back into the original container.
- Spillages should be wiped up as they occur to avoid cross-contamination of components.
- A check on the pH value of the medium should be carried out prior to and after autoclaving, as this can indicate if errors have been made during preparation.
- All other controls should be as for dehydrated media (for details refer to previous sections).

Appendix 1
Trouble Shooting Guide

PROBLEM	POSSIBLE CAUSE(S)
Abnormal Colour	Use of impure water or dirty glassware. Too harsh a heat treatment applied. Deterioration during storage of medium. Incorrect pH.
Coagulation	Supplements added to medium which has not sufficiently cooled
Darkening	Too harsh a heat treatment - particularly in media with a high carbohydrate content.
Flecks in media	Black - Charring of media Clear - Opaque supplements added to media which have cooled to the extent that they are beginning to set at the sides of the container.

GUIDELINE NO. 6, 11/96, updated 12/03: THE MAINTENANCE AND HANDLING OF REFERENCE ORGANISMS

Introduction

This guideline document should be used in conjunction with EA-04/10, Accreditation for Microbiological Testing (available at www.european.accreditation.org). This gives guidance on suitable sources and methods of maintenance of reference organisms. The aim of this document is to outline further, the options available and to give practical guidance on associated good practices.

Reference cultures

Recognised sources of microbiological reference culture collections are listed in Appendix 1 of this Guideline.

Where reference cultures are supplied in a form which necessitates reconstitution e.g. freeze-dried ampoules, they should only be sub-cultured once, to produce reference stocks. An assessment should be made of the purity of the culture at this stage and where appropriate, verification that the strain has the required characteristics (alternatively the latter can be assessed as the working culture is being used).

The frequency of renewal of reference cultures will depend on a number of factors, including the maintenance procedures used, storage space available and the fragility of the strains in question. Recommendations should be substantiated by in-house experience.

Reference stocks

Strains can be maintained as reference stocks by a number of means. Examples include:

a) storage under liquid nitrogen
b) freeze-drying
c) freezing at temperatures below -20°C (either in liquid broth or on beads).

Reference stocks must be replenished through receipt of fresh reference cultures; working cultures *must not* be used to regenerate reference stocks.

Working cultures

Working cultures are cultures derived from reference stocks. Working cultures are for use in the laboratory on a day-to-day basis.

Organisms are commonly held as working cultures either in broth suspension, or on agar slopes, for a defined period of time. For fastidious organisms, or in situations of

infrequent use, it may be more appropriate to use cultures directly from reference stocks, discarding the culture after use. Alternatively, the use of specific enrichment media, e.g. cooked meat broth for anaerobes, can be used to maintain some of the more fastidious organisms.

To minimise problems of contamination with repeated use of individual cultures, it is advisable to prepare several working cultures at the same time, for use over different time periods.

If further subculture of working cultures is to be performed, it must be kept to a minimum and be supported by appropriate morphological and biochemical checks to demonstrate that loss of viability, biochemical activity or morphology does not occur.

Alternative culture sources

Laboratories may choose to purchase strains from sources other than the recognised culture collections, or in a form other than freeze-dried ampoules. In such instances full traceability to an appropriate National Collection should be demonstrated and the product must be shown to be produced under a relevant quality system, i.e. BS EN ISO 9001:2000, Quality management Systems and associated standards (see PFMG AAG Guideline No. 4, Quality assurance of laboratory consumables). It is recommended that supporting information relating to organism traceability and the production QC procedures involved is also obtained from the manufacturer.

Documentation

Details to be kept of the cultures received together with the maintenance and use procedures employed must be fully documented in a Standard Operational Procedure, supported by comprehensive records to demonstrate that this is being followed.

British Standards are available from the British Standards Institution, Customer Services, 389 Chiswick High Road, London W4 4AL, UK

APPENDIX 1: Sources of cultures of reference microorganisms

1. National Collection of Type Cultures (NCTC), Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT.

2. National Collection of Pathogenic Fungi (NCPF), Public Health Laboratory, Mycological Reference Laboratory, Myrtle Road, Kingsdown, Bristol, BS2 8EL.

3. CABI Bioscience (formerly the International Mycological Institute (IMI)), Bakeham Lane, Egham, Surrey TW20 9TY.

4. National Collection of Yeast Cultures (NCYC), Institute of Food Research, Colney Lane, Norwich, Norfolk NR4 7UA.

5. National Collections of Industrial, Food and Marine Bacteria Ltd (NCIMB) 23 Machar Drive, Aberdeen AB24 3RY.

6. Culture Collections of Algae and Protozoa (CCAP), Dunstaffnage Marine Laboratory, PO Box 3, Oban, Argyll PA34 4AD, Scotland.

6. Culture Collection of Algae and Protozoa (freshwater) (CCAP), Institute of Freshwater Ecology,The Ferry House, Far Sawrey, Ambleside, Cumbria LA22 0LP.

7. European Collection of Cell Cultures (ECACC), Health Protection Agency, Porton Down, Salisbury, Wiltshire SP4 0JG.

8. Central Bureau Voor Schimmelcultures (CBS), PO Box 273, Oosterstraat 1, NL-3740 AG Baarn, Netherlands.

9. American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md 20852, USA.

10. Microbial Strain Data Network - an International Network of Microbial and Cell Line Information Resources, Institute of Biotechnology, Cambridge University, 307 Huntingdon Road, Cambridge CB3 0JX.

11. Information Centre for European Culture Collections, Mascheroder Weg 1b, D-3300 Braunschweig, Germany.

Comprehensive information is available from the website of the United Kingdom National Culture Collection (www.ukncc.co.uk)

GUIDELINE NO. 7, 10/96, updated 12/03: TEMPERATURE MONITORING

Introduction

This guideline should be used with reference to the following documents.

EA-04/10. Accreditation for Microbiological Laboratories (available from www.european-accreditation.org)

British Standards:

BS 593 (1989:confirmed) Specification for Laboratory Thermometers
BS 1041-2.1 (1985) Guide to selection and use of liquid-in-glass thermometers.
BS 1041-3 (1989) Guide to selection and use of industrial resistance thermometers.
BS 1041-4 (1992) Guide to the selection and use of thermocouples.
BS EN ISO/IEC 17025 (2000) General requirements for the competence of testing and calibration laboratories.

British Standards are available from the British Standards Institution, Customer Services, 389 Chiswick High Road, London W4 4AL, UK

United Kingdom Accreditation Service (UKAS) documents:

LAB11.Traceability of Temperature Measurement
LAB12. The Expression of Uncertainty in Testing
M3003. The Expression of Uncertainty and Confidence in Measurement.
UKAS Directories of accredited organisations (www.ukas.com)

UKAS documents are available from the United Kingdom Accreditation Service, 21-47 High Street, Feltham, Middlesex TW13 4UN, UK

It is important to ensure that thermometers are sufficiently accurate, easy to read and have an acceptable uncertainty of measurement. Thermometers must be used according to the manufacturer's instructions paying particular attention to immersion requirements, details of which are obtainable from the manufacturer, i.e. partial or total immersion, as well as to the orientation (vertical or horizontal) of the thermometer. The advantages and disadvantages of the different thermometer types are detailed in Appendix 1 of this Guideline.

Calibration

a) Reference Thermometers

Reference thermometers are temperature measuring devices used by a laboratory to calibrate other, (working), thermometers.
Calibration of all reference temperature devices should be carried out by an accredited **calibration** laboratory, see UKAS Directories of accredited organisations or, the National Physical Laboratory (NPL) and a valid calibration certificate obtained.

i) Reference Liquid-in-Glass Thermometers

Re-calibration should be carried out at least once every 5 years.
The ice point should be checked at least once a year, either in-house following the procedure in BS1041-2.1 Appendix C, or by an accredited calibration laboratory.

Ii) Reference Thermocouples

Re-calibration interval should not exceed 4 years. Specific intervals cannot be given, but the closer the temperature is to the maximum recommended in BS 1041-4:1992, and the more frequently the thermocouple is used, the shorter the re-calibration interval should be.

iii) Reference Platinum Resistance Thermometers (PRT's)

Reference PRT's should be checked at the ice or triple-point before use. The triple point is the point at which all three states of water co-exist i.e. ice, water and water vapour. By definition the triple point for water is achieved at 273.16K. In practice it appears as a 'slush' ice/water mix. Re-calibration should be carried out at intervals depending upon the frequency and temperature of use, but should not exceed 5 years. (See BS 1041-3).

b) Working Thermometers

Working thermometers are defined as thermometers that are in every day use and have been checked against a reference thermometer.

When selecting a working thermometer ensure that the thermometer is capable of measuring accurately within the range required. Avoid unnecessary expenditure on working thermometers with an accuracy greater than is required for the analysis.

i) Working Liquid-in-Glass

Where the accuracy of temperature measurement has a significant effect on the test result a valid calibration certificate should be held for the

thermometer. Alternatively calibration may be carried out in-house against a reference thermometer (BS 1041-2.1).

Where a temperature measurement is specified in the test procedure but its accuracy does not have a direct bearing on the test result, then a BS 593 standard thermometer may be used. This thermometer should be re-calibrated after 5 years as indicated in BS 593. BS 593 thermometers may be used where the temperature has a significant effect on the test result if calibrated to the NPL standard.

On receipt of a new thermometer, a calibration check should be carried out over a range of temperatures including the working temperatures and reference point, e.g. ice point; if the ice point is not marked on the thermometer the lowest point on the

Thermometer should be used. Due to stabilisation of the bulb volume, reference point checks are required at 6 monthly intervals in the first year and once a year thereafter. Re-calibration should be carried out every 5 years, or sooner if the checks at the reference point indicate a significant change.

Total immersion thermometers are intended for use in closed vessels e.g. incubators, ovens and autoclaves. Partial immersion thermometers are intended for use in waterbaths.

ii) Working Thermocouples

Working thermocouples should be calibrated either in-house, against a reference thermometer or by a UKAS accredited calibration laboratory. Liquid-in-glass thermometers or thermocouples may be used as reference thermometers. The intervals between successive calibrations will depend upon the application and range of temperature measurement of the thermocouple. The maximum interval should not exceed one year.

iii) Working Platinum Resistance Thermometers (PRT)

Working PRT's should be calibrated either in-house, against a reference thermometer or by a UKAS accredited calibration laboratory. Liquid-in-glass thermometers or PRT's may be used as reference thermometers. The intervals between successive calibrations will depend upon the application and range of temperature measurement of the thermometer. The maximum interval should not exceed one year. A check at the ice or water triple point shall be carried out at least once a year.

Tolerances

Tolerances should be set in the test or equipment operating procedure and should take into account the uncertainties of measurement associated with the reference thermometer as quoted on the calibration certificate. An example of how to calculate the total uncertainty of measurement is shown below.

Reference thermometer uncertainty of measurement = ± 0.12°C

Working thermometer uncertainty of measurement = ± 0.2°C

Total uncertainty of measurement = $\sqrt{(0.2^2 + 0.12^2)}$ = ± 0.23°C

To obtain a working tolerance for the test, subtract the total uncertainty of measurement from the temperature tolerance described in the test procedure. e.g.

Analysis tolerance = 37°C ± 0.5°C
Total uncertainty of measurement attached to measuring device = ± 0.23°C

Working analysis tolerance = 37°C ± (0.5°C - 0.23°C) = 37°C ± 0.27°C

Labelling

Reference and working thermometers should be labelled with the following.

a. Date of last calibration.
b. Date of next calibration.
c. Date of last reference point check.
d. Date of next reference point check.
e.

If the thermometer is marked with a unique identification number then this information may be recorded in a logbook referring back to the unique number.

Documentation

Procedures relating to calibration and checking of thermometers should be fully documented and all relevant data recorded.

Appendix 1: Advantages and Disadvantages of Reference and Working Thermometers.

Thermometer Type	Advantages	Disadvantages
Liquid in glass	Cheap Easy to use	Easily breakable Mercury toxicity Small range For total immersion thermometers the measuring point is within the incubator / waterbath etc.
Thermocouple	Relatively cheap Relatively accurate Easy to read Large range Measuring point remote from incubator / waterbath etc. Insulated types rarely affected by vibration / shock	Susceptible to electrical interference Variable long term stability
Platinum resistance	Good long term stability Good accuracy Rarely susceptible to electrical pick-up Measuring point remote from incubator / waterbath etc.	Relatively expensive Susceptible to vibration / shock Unsuitable for high temperatures

GUIDELINE NO. 8, 6/98, updated 12/03: EQUIPMENT CALIBRATION

Introduction

Laboratory equipment plays an important part in the conduct of microbiology tests and can contribute to the reliability of the test results generated. Key factors to consider in relation to laboratory equipment include:

- equipment should be of appropriate accuracy and be suitable for the intended use
- equipment should be properly commissioned and maintained in good working order thereafter
- equipment should be cleaned and serviced regularly
- equipment should be calibrated and checked for continuing accuracy at regular intervals.

Although each of these factors is important, this Guideline concerns equipment calibration and checks for monitoring performance accuracy. Calibration and subsequent monitoring checks help demonstrate that the equipment used functions properly and within parameters defined by the test method or equipment specification. This Guideline document should be used in conjunction with:

- The equipment manufacturer's information.

- EA-04/10. Accreditation for Microbiological Laboratories (available from www.european-accreditation.org)

- British Standards:
  BS EN ISO/IEC 17025 (2000) General requirements for the competence of testing and calibration laboratories.

  British Standards are available from the British Standards Institution, Customer Services, 389 Chiswick High Road, London W4 4AL, UK

- UKAS Directories of accredited organisations (www.ukas.com)

UKAS documents are available from the United Kingdom Accreditation Service, 21-47 High Street, Feltham, Middlesex TW13 4UN, UK

Calibration and Monitoring Checks

General requirements

Primary instrument / equipment calibration is the responsibility of the manufacturer and is carried out to ensure that performance specifications are met. It is the user laboratory's

responsibility to select for use, appropriate validated methodology supported by the use of appropriately calibrated and monitored instrumentation and equipment.

A laboratory should operate a defined programme for calibrations, tests and supporting / subsidiary measurements. Where practically possible, these should be traceable to National Standards of measurement. Where this is not possible, the laboratory should operate a suitable programme of measurement audit e.g. inter-laboratory comparison or regular use of Standard Reference Materials so that satisfactory evidence of correlation of calibration or test procedures can be demonstrated.

Provided that a laboratory can adequately demonstrate the correct functioning of equipment i.e. its suitability for purpose, there is no requirement to use external calibration agents (third parties) unless it is not possible to carry out the necessary calibrations or monitoring checks to the required standards by 'in-house' means.

Calibration and monitoring checks carried out in-house

If a laboratory carries out any calibration and / or accuracy monitoring check in-house, then fully documented procedures detailing the practical conduct of these tests must be held together with full records of the work done and results obtained.

Suitable reference materials will need to be held and these must be traceable to National Standards (where applicable). Whenever possible, the reference materials should be calibrated by the relevant national service e.g. National Physical Laboratory or an appropriate UKAS accredited organisation.

Reference materials e.g. thermometers and weights, used to perform in-house calibrations must be used solely for calibration purposes. They should be more accurate than the test equipment they are used to calibrate. To ensure their continued accuracy, they must be re-calibrated at defined intervals and full records must be held of these events. Where relevant, appropriate correction factors derived from the calibration certificate will need to be taken into account when used to calibrate laboratory equipment.

Calibration and monitoring checks carried out by a third party

If a laboratory employs the services of a third party to carry out equipment calibrations and / or monitoring checks, wherever possible, it is recommended that a UKAS accredited service is used. Details of accredited calibration organisations are available from UKAS. The laboratory should ensure that all the required calibrations / checks are done and that full, complete, clear and unambiguous records are obtained from the third party. Where applicable, the reference materials used by the third party must be traceable to National Standards. Records to demonstrate this should also be held by the laboratory.

For laboratories seeking accreditation from UKAS, the particular requirements of the British Standard BS EN ISO/IEC 17025 (2000) must be observed in relation to third party (external) agents and the traceability of any test equipment used.

Application of correction factors

A laboratory must have a documented policy indicating what errors are considered significant for each type of equipment and the action to be taken to ensure that any appropriate correction factors are applied when equipment is used.

Maintenance of essential equipment should be carried out at regular intervals and detailed records maintained. Essential equipment normally found in food examination laboratories are; incubators, water baths, balances, microscopes, autoclaves, thermometers, hygrometers, centrifuges, pH meters, refrigerators, freezers, automatic colony counters, automatic pipetters, diluters and dispensers.

Some equipment will need to be calibrated regularly e.g. autoclaves, thermometers and balances, as well as requiring monitoring checks in-between calibrations. Re-calibration will also be necessary after repair or modification of equipment. However, for most equipment, regular maintenance and performance checks should be carried out to ensure that equipment meets the stated / required performance specification.

IT IS THEREFORE ESSENTIAL THAT A PERFORMANCE SPECIFICATION IS STATED BY AND OBTAINED FROM THE MANUFACTURER.

Examples of Calibration and Monitoring Checks for equipment most commonly used in a food microbiology laboratory are given in Appendix 1 of this Guideline. Other examples can be found in CCFRA Guideline No. 9: (1996) 'A Code of Practice for Laboratories Handling Food Samples' (Campden & Chorleywood Food Research Association, Chipping Campden, UK) and EA-04/10 Accreditation for Microbiological Laboratories.

Calibration / Monitoring Checklist

1) Does the equipment have a performance specification or are the parameters for its correct operation defined by the test method?

2) Are the performance specification or defined tolerances acceptable and realistic / suitable for the proposed use?

3) Does the equipment need calibration and / or monitoring checks?

4) What calibration or monitoring checks are required?

5) Is traceability applicable and is there a traceable National Standard?

6) What reference material should be used, how often should it be calibrated and are records of traceability held in the laboratory?

7) What frequency is appropriate for the calibration or monitoring check?

8) Are records of the calibration or monitoring checks held and, are they up to date?

9) Should account be taken of any errors, if so, what correction factors should be applied? Have they been correctly identified and applied?

10) Are controls in place to ensure that 'out of calibration / specification' equipment is not used?

11) Are the equipment documentary systems complete and up to date including, manufacturer's information (specification), commissioning data and report, standard operating procedures, calibration / monitoring procedures, maintenance and repair reports?

Documentation

Records must be maintained clearly and correctly and kept up to date.
All documents and records relating to any aspect of calibration must be kept for an appropriate period of time.

Appendix 1

Note - this information is provided for guidance purposes only

EQUIPMENT CALIBRATION AND MONITORING					
Item	Measurement	Calibration / Monitoring Reference Method	Suggested Tolerance	Suggested Frequency	AAG Guideline No.
Autoclave: Calibration	Temperature, time	Service and Calibration to BS 2646 Part 1 (1993) and Part 5 (1993). UKAS accredited organisation recommended		Annually	2
Monitoring	Temperature	Calibrated maximum thermometer	± 1°C of target temp	Each use	7
	Temperature, time	Calibrated thermocouple / chart recorder	± 1°C, ± 2 min	Each use	
	Process	Colour change devices or biological test strips	Must meet test specification	Each use	
Balance: Calibration	Weight	Calibration to National Standard (UKAS accredited organisation recommended)	As appropriate for limits of machine's accuracy	Annually	3
Monitoring	Weight	Known weight(s) in working range	± 1% } of target weight or ± 1% } as appropriate to use	Daily	
		Linearity checks using known weights over the required working range		Weekly	
Conductivity Meter	Conductivity	Appropriate standard solution(s) of known conductivity	Within tolerances specified by meter manufacturer	As per manufacturers' instructions	
Diluters	Volume	Weight of water in working range using calibrated balance	± 2% of target volume or as appropriate to test requirements	Each use	
Dispensers	Sterility	If appropriate, sterility check using non-selective medium	Sterility	Each use	
Incubators	Temperature	Dedicated calibrated thermometer / temperature monitoring device mounted in a suitable heat sink e.g. glycerol; see also, 'Thermometers/Temperature monitoring devices' below	As appropriate to test e.g. ± 1°C or ± 0.5°C of target	Daily	7
pH Meters:	pH value	2 reference buffers, in the expected range of use e.g. pH 4 to 7. Temperature compensation allowances should be made	± 0.1 pH unit or as appropriate to the test	According to the stability of the meter e.g. daily or per use	9 Part I
Pipettes: - non-automatic	Volume	Weight of water using calibrated balance	As test requirement or ± 2% of target volume	Proportion of each batch delivered	3
- automatic	Volume	Weight of water using calibrated balance	As test requirement or ± 2% of target volume	Daily or weekly as appropriate, also after any dismantling / cleaning	3

EQUIPMENT CALIBRATION AND MONITORING					
Item	Measurement	Calibration / Monitoring Reference Method	Suggested Tolerance	Suggested Frequency	AAG Guideline No.
Refrigerators / Freezers	Temperature	Dedicated calibrated thermometer / temperature monitoring device mounted in a suitable heat sink e.g. glycerol; see also, 'Thermometers / Temperature monitoring devices' below	To specified range required for application	Daily	7
Laminar air- flow and Safety Cabinet	Filter integrity, air flow rate In use sterility check	Maintain according to the manufacturer's recommendations. Refer also to BS5726-4 (1992) Microbiological safety cabinets. Recommendations for selection, use and maintenance. Microbiological agar plate exposure	As specified by manufacturer No growth	Minimum annually or appropriate to use Each use	
Sterilising oven	Temperature and time	Calibrated thermometer / thermocouple and calibrated timer; see also, 'Thermometers / Temperature monitoring devices' below	±2°C of the minimum temperature required e.g. ±2°C of 170°C for the required time	Daily / Each use	7
Thermometers / Temperature monitoring devices: - Reference	Temperature accuracy	Calibration to National Standard over the range of temperatures appropriate to use	Apply correction factor	Every 2 years or as appropriate to use	7
- Working: a) most critical (± 0.5°C)	Temperature accuracy Temperature accuracy	Calibration to National Standard (i) BS 593 thermometer, or as a) for 0.5°C	Apply correction factor if significant to use Apply correction factor if significant to use	Annually Annually	7 7
b) critical (± 1°C) c) least critical (± > 1°C)	Temperature accuracy	(ii) Thermometer / device checked against a) or b) type devices	Apply correction factor	Annually	7
Timers:	Time	Speaking clock / National Time Signal	As appropriate to use	As appropriate to use	
Water bath:	Temperature	Dedicated calibrated thermometer / temperature monitoring device; see also, 'Thermometers / Temperature monitoring devices' above	As appropriate to test e.g. ± 1°C or ± 0.5°C of target	Daily	7
Water purifiers:	Conductivity	Conductivity meter	Less than the maximum specified for laboratory application	Daily	
Weights: - Reference: - Working:	Weight Weight	Calibration to National Standard 1. Calibrated weight 2. Known weight measured on a calibrated balance (keep protected)	 Apply correction factor if significant to use	Annually Annually	

British Standards are available from the British Standards Institution, Customer Services, 389 Chiswick High Road, London W4 4AL, UK

GUIDELINE No. 9,4/97, updated 12/03
PART I : RECOMMENDED CHECKS FOR pH METERS

Introduction

This guideline document describes the routine checks that should be made on pH meters and electrodes. The measurement of pH involves the use of two half cells, a hydrogen cell and reference cell, generally combined as combination electrodes and should be used in conjunction with the manufacturer's instructions and with reference to the following documents:

- United Kingdom Accreditation Service (UKAS) document:
  LAB11.Traceability of Temperature Measurement
  UKAS documents are available from the United Kingdom Accreditation Service, 21-47 High Street, Feltham, Middlesex TW13 4UN, UK
- PFMG AAG Guideline 5 : Media storage and preparation.
- PFMG AAG Guideline 8 : Equipment Calibration
- PFMG AAG Guideline 9 : Part II: Measuring Media pH values

The pH value of a medium is defined as the logarithm to the base ten of the reciprocal of the hydrogen ion concentration.
$pH = \log_{10} 1/[H^+]$.

It is important to ensure that the pH meter is sufficiently accurate for the pH tolerances being monitored in media. It is recommended that an accuracy of +/- 0.02 is obtained. This is the accuracy to which buffer solutions are generally manufactured. Where there is no automatic temperature compensation, a thermometer is required. It is recommended that a pH meter with in-built automatic temperature compensation is purchased which reads to 2 decimal places (1 decimal place may suffice).

Calibration of pH meter

The pH meter must be calibrated at a minimum frequency of **daily**, using known buffer solutions. As the stability of pH meters may drift over time it is recommended that regular checks on the calibration status be made between calibrations using a buffer that is appropriate to the pH range of the expected measurements, re-calibrating when significant (refer to stated tolerance) drifting occurs.

a) **Solution Temperature Values**

The pH of all buffer solutions varies with solution temperature. For accurate calibration it is necessary to measure the temperature of the buffer solution being used prior to calibration. Manufacturers of buffer powders and solutions will provide a table of values for their buffers at varying temperatures. Normally measurements are taken at room temperature (20-25°C).

b) **Buffer Solutions**

Refer to the manufacturer's instructions for calibration of the pH meter. During calibration, ensure that the buffer solution is stirred gently and that a stable readout on the display is achieved prior to and following any adjustment.

Buffer solutions must be fresh. These should be replaced regularly. Care must be taken in making up buffer solutions i.e. tablets must be completely dissolved and the correct volume of (deionised or distilled) water used. It is recommended that ready prepared buffer solutions that are certificated to traceable standards be purchased. Where ready prepared buffers are used, the stock solution must be kept separately from the working solution. Buffer solutions can become contaminated when exposed to air and should be stored in sealed containers and regularly replaced. Used solutions should be discarded after a week.

The pH meter is calibrated using 2 buffers pH4 and pH7 or pH7 and pH10, which must reflect the working range of the expected pH measurements. The pH of the buffers must be measured as stated by the manufacturer e.g. 20 or 25°C unless there is automatic temperature compensation. The buffer should be kept gently stirred during calibration (***care***: air bubbles affect the reading.).

- Immerse the electrode in the buffer pH7.
- Allow to stabilise.
- Set the display to 7.0 using the buffer control.
- Repeat with at least one other buffer.
- As necessary, adjust displayed value slope control (following manufacturer's instructions)
- A stable read out on the display must be achieved prior to and following any adjustment.

Selection of Electrodes

There are several types of pH electrode and it is important to select the type which is most appropriate for the application. Factors to consider include;-

- separate or combined glass and reference electrodes
- the composition of the electrode body e.g. glass or epoxy resin
- the shape of the pH bulb
- the design of the measuring head
- speed of response
- the types of media / solution on which determination of pH is required.

Consult the manufacturer to ensure the correct electrodes are supplied.

Care and Checking of Electrodes

The sensitive glass pH membrane or reference junction should not be touched during use. Excess droplets of solution may be removed by gently tapping the electrode body. Larger bubbles may be removed by shaking the electrode in a downward direction. Ensure that the side port / inlet is uncovered, especially during a long run of tests. A flat tip Gel plas Epoxy Body sealed reference combination electrode eliminates the need to add filling solutions. Otherwise, ensure the level remains above the sample level in use.

Electrodes and pH meters are delicate instruments. They must be kept clean and in good condition and used carefully. Follow the manufacturer's instructions for advice on storage of the electrodes. Always ensure the electrode is used within its specific temperature range. Electrodes used above their specified temperature range undergo rapid and irreversible ageing.

a) **Storage**

Storage conditions will vary for different electrodes and the manufacturer's instructions should always be consulted. General factors that should be borne in mind include:-

- Always rinse electrodes after use e.g. with distilled or deionised water
- Store electrodes in a vertical position, away from direct sunlight and within their specified temperature range.
- Ensure that the electrodes are kept moist and stored in the correct storage solution specified by the manufacturer e.g. aqueous potassium chloride.

b) **Cleaning**

Great care is needed when cleaning glass bulbs. During use the electrode must be rinsed with distilled or deionised water between each measurement to eliminate contamination between solutions. Daily cleaning is recommended by wiping after use. Monthly enzymatic cleaning is recommended for protein contamination. Ethanol swabs may be used to remove fat. Acid/alkali cleaning might be required (see manufacturer's instructions).

Tolerances

Accuracy of a pH meter depends upon the specification, the accuracy of the scale reading on the pH meter and the buffer solutions available. Refer to manufacturer's manual.

Documentation

All procedures relating to electrode maintenance, calibration and use of pH meters must be fully documented. Adequate data must be maintained to show when:

a) buffer solutions were prepared,
b) electrodes were entered into use and
c) the pH meter was last calibrated.

GUIDELINE NO. 9, 4/97, updated 12/03
PART II: MEASURING MEDIA pH values

Introduction

The pH value of the local environment is important to the survival and rate of growth of microorganisms. Ensuring that microbiological media is of the required pH value is therefore an important consideration in the recovery and enumeration of micro-organisms. For organisms that grow only within a restricted pH range, the medium must be at the pH value that is optimal for their growth.

This guideline (part II) should be used in conjunction with PFMG AAG Guideline No.9 Part I - Recommended checks for pH Meters.

It is important that the pH meter used is of sufficient accuracy for the intended use. Most media manufacturers specify pH value tolerances of +/- 0.2 for media pH. In view of this a meter having an accuracy of +/- 0.1 pH unit would be suitable for measuring media pH, however, for more fundamental measurements of pH value, it is preferable that a meter with an accuracy of +/- 0.02 is used.

General

Owing to the low ion concentration in water the addition of 0.3ml saturated potassium chloride to 100 ml water is necessary to measure the pH value of pure water as is difficult to measure accurately and requires a specialised electrode system.

Water stored in polythene containers tends towards an acid pH. Boiled and subsequently cooled water alters an acid pH towards neutral. Water conductivity should preferably be used as a measure of the quality of the water to be used in microbiological work.

Unless otherwise specified, media pH ranges given on the manufacturer's product labels refer to final and complete cooled medium i.e. supplements must be added before pH determination.

Wherever possible similar volumes of media should be processed together to minimise the risk of over heating which can lead to denaturation and / or pH changes of the medium.

Electrodes

It is essential that the electrode used is suitable for the intended purpose. There are a number of pH electrodes available and the choice will depend upon a number of factors including the ionic strength of the solutions to be measured. Maximum Recovery Diluent, Ringers solution and water in particular, are low in ion concentration. Standard

laboratory electrodes may therefore be unsuitable for the accurate measurement of pH for these liquids.

For measuring the pH value of gels and set agar the use of a Gel Electrode may be beneficial.

Cleaning

Refer to AAG Guideline 9 Part I : Recommended checks for pH Meters.
When electrodes are used for media they are required to be cleaned regularly to avoid accumulation of protein and blood deposits. Soaking the electrode in a solution of 1% Pepsin in 0.1 M HCI for 4 hours removes deposits.

Measurement of pH

The pH value of each laboratory prepared batch of media should be checked and recorded. The pH should be determined on cooled media after autoclaving (or boiling for heat sensitive media), after the addition of any supplements. The pH value of media containing gelatin may be measured more conveniently if liquefied at 37°C and using a meter with an in-built temperature compensation system.

Routine Checks

Prior to using a pH meter, check:

a) batteries are charged if the meter is battery powered
b) sufficient time is allowed to stabilise the instrument after switching on if mains connected
c) buffer solutions are fresh and within expiry date
d) calibration is achieved with stable readings.

Tolerances

Media tolerances for pH value are clearly specified by each manufacturer. The pH range of media is based both on historical and manufacturer's data for single batches within autoclave loads of the same media types and volumes. Problems may arise with mixed batches. (Autoclave time may need to be adjusted accordingly and validation data obtained.)

The pH value of the prepared medium must fall within the manufacturer's specified range. It should only be used if sufficient quality monitoring information has established that the organism for which it is intended can be fully recovered on the medium and the supportive data can verify this e.g. by Relative Growth Index methods.

The acceptable pH tolerances stipulated by media manufacturers are usually ±0.2 pH unit. This assumes constant 1-litre volumes produced strictly according to media manufacturer's instructions.

Adjustment of media pH should not be necessary if all systems are correct e.g. water quality, volume of water, weight of powder and the sterilisation process are all in accordance with the manufacturer's instructions.

If the pH of a medium needs to be adjusted, titration of N/10 HC1 or N/10 NaOH into sample aliquots of media should be carried out. The sample media should then be processed, and the pH measured on cooling and after addition of any supplements. Calculations for pH adjustment of the larger batch can then be made.

Effect of pH

Autoclaving or irradiation may lower the pH of a medium by about 0.2 - 0.5 units.

Culture media reactions are affected by pH;

If too ACIDIC	a)	Bile salts precipitate
	b)	H_2S reactions depressed
	c)	Interferes with sugar reactions
	d)	Decrease in activity of some antibiotics e.g. aminoglycosides. cephalosporins, macrolides
If too ALKALINE	a)	Potentiates aminoglycosides
	b)	Interferes with sugar reactions
	c)	Decrease in activity of some antibiotics e.g. fusidin, tetracycline, penicillins.

The low ionic strength of Ringers and Maximum Recovery Diluent cause difficulty in pH measurements therefore, conductivity measurement would be more relevant where media manufacturer's provide such information.

Documentation

Procedures relating to the conduct of pH checks on media must be documented and tolerances for each medium pH defined. Records of all pH checks must be held. Any out of specification pH values must be highlighted and the affected media should only be used if appropriate growth validation checks have been conducted on the media to demonstrate its performance is still acceptable.

Quality Control using microbiological criteria is essential.

References and further reading

Oxoid Manual - 8th Edition 1998

PFMG- AAG Guideline 9 Part I : Recommended Checks for pH Meters.

Helmuth Galser (1991) : pH measurement, fundamentals, methods, applications and instrumentation. VCH Publishers.

GUIDELINE No. 10, 4/97, updated 12/03: RECOMMENDED CHECKS FOR WATER ACTIVITY INSTRUMENTS

Introduction

This guideline document should be used in conjunction with the appropriate manufacturer's instructions and with reference to the following documents:

- EA-04/10. Accreditation for Microbiological Laboratories (available from www.european-accreditation.org)
- UKAS Directories of accredited organisations (www.ukas.com)
- The Humidity Code of Practice. The Institute of Measurement & Control.

Definitions

Water activity (a_w) is the ratio between the partial water vapour pressure at the surface of the sample (p) and the partial pressure of pure water at the same temperature (p_o):

$$a_w = \frac{p}{p_o}$$

Water activity is indirectly obtained from a measurement of the equilibrium relative humidity (ERH) reached in a closed system at constant temperature. The relationship between water activity and ERH is given by the formula:

$$a_w = \frac{ERH}{100}$$

CALIBRATION OF INSTRUMENTS

Introduction

Standard salts are used to calibrate measuring instruments. Normally these are water-saturated salts whose ERH at a given temperature is known (to ± 0.3%). These solutions may easily be prepared in the laboratory. Normally, readings are taken at 25 ± 0.2°C, which is in line with European regulations (European Directive 77/99/CEE, article 5).

Preparation of standard solutions

The following table lists examples of salts and their ERH at 25°C.

Salt	Formula	ERH (%)	Quantity of salt (g)	Quantity of water (g)
Lithium chloride	LiCl	11.3	50	10
Potassium acetate	CH_3COOK	22.5	50	10
Magnesium chloride	$MgCl_2.\ 6H_2O$	32.8	40	5
Potassium carbonate	K_2CO_3	43.2	50	10
Magnesium nitrate	$Mg\ (NO_3)_2.6H_2O$	52.9	60	10
Sodium bromide	NaBr	57.7	50	10
Strontium chloride	$SrCl_2.6H_2O$	70.9	40	5
Sodium chloride	NaCl	75.3	40	10
Potassium chloride	KCl	84.3	40	10
Barium chloride	$BaCl_2.2H_2O$	90.2	40	5
Potassium sulphate	K_2SO_4	97.3	50	10
Potassium dichromate	$K_2Cr_2O_7$	98.0	50	10

The most commonly used solutions are lithium chloride, magnesium nitrate and potassium chloride, but for most accurate calibration, salts of an ERH that most closely match that of the sample should be used. A minimum of two salts but preferably three must be used to calibrate over a range.

To prepare the solutions, hydrate the chosen salt in the proportions indicated, in a flask with a stopper. Ensure that the salt is well moistened and mix until a thin supernatant appears. Allow this solution to stabilise at 25°C for 2 - 3 hours before use. The standard salt solutions should be stored in air-tight flasks, with a thin supernatant to ensure the correct humidity is maintained and also to prevent drying out by evaporation. Store these at 25°C ± 1°C, (either in a room or a temperature-controlled incubator).

Methods of calibration

In-house

Methods of calibration differ according to the instrument, and the instrument manual should be consulted. Calibration should be done at three points, to allow for a non-linear response of the sensor. The instrument (as well as the standards and samples) must be stabilised at the measuring temperature e.g. for 12 hours or overnight. It is advisable to re-calibrate the instrument on at least a monthly basis and suitable records must be kept showing that this has been done.

UKAS Accredited Calibration

Water activity instruments can be calibrated to recognised standards, in one of three ways:

- by the user applying traceable salt standards ("transfer standards")
- by sending the instrument to a suitable UKAS accredited calibration organisation
- on-site, by the manufacturer using their own UKAS transfer standards.

GENERAL PROCEDURES FOR DETERMINATION OF WATER ACTIVITY

As a guide to expected water activity (and hence the standards to use), the normal water activity of food products can be grouped as follows:

Food product	Water activity
Dehydrated products	0.05 - 0.45
Intermediate moisture products	0.40 - 0.75
Pasty or liquid products	0.70 - 0.98

Check the calibration of the instrument using the appropriate standard salts.

Measure the ERH of the test portion, allowing sufficient time for the instrument to stabilise. Place the sample quickly and cleanly into the sensor chamber to avoid any moisture uptake or loss. Avoid touching the sensor head, as this will invalidate the result. Store any remaining product in an air-tight container with as small a headspace as possible. Normally, duplicate determinations of ERH should be carried out (using separate sample portions).

After each measurement of ERH that exceeds 80%, dry the measuring chamber and the sensor using regenerated silica gel or a molecular sieve.

The difference between two single test results obtained with the same method on the same sample obtained within a short interval of time should not exceed 0.5% ERH (0.005 a_w).

FACTORS THAT COULD AFFECT DETERMINATION OF WATER ACTIVITY

The following factors may influence the determination:

- temperature: *make measurements at 25℃ ± 0.2℃*
- volatiles: certain volatile materials are harmful to the sensors, e.g. propylene glycol, fatty vapours, sorbitol, glycerol, acetic acid, alcohols, ammonia: *use a chemical filter to protect the sensor*
- contamination of the sensor: *keep the sensor clean*

- mechanical shocks and air draughts: *keep equipment on a solid platform or bench away from draughts*

- condition of the sample; non-homogeneous samples e.g. jams with fruit chunks, cakes containing fruits and nuts, and ready-made meals can take a long time to equilibrate to one ERH value: *allow sufficient time to obtain reliable results*

- equilibrium with the sensor; samples containing significant quantities of oils and fats e.g. butter, cream, salad dressings and mayonnaise may take a long time to equilibrate: *allow sufficient time to obtain reliable results*

- Degree of airspace: *keep headspace to a minimum*

Records

The following records must be kept (where relevant)

- details of servicing and repairs to the instrument
- in-house calibrations using traceable salt solutions
- appropriate calibration certificates for the instrument / humidity salts.

GUIDELINE NO.11, 4/97, updated 12/03:
RECOMMENDED CHECKS FOR ELISA WASHER & READER EQUIPMENT

Introduction

Enzyme Linked ImmunoSorbent Assay (ELISA) is a labelling technique for demonstrating the presence, or absence of an antigen or antibody. The method depends on the use of one of these immunoreagents, coupled to an enzyme (this is known as a conjugate). A step to separate free and bound conjugate is then necessary to obtain a result.

The ELISA technique has been used in clinical laboratories since 1976. From the mid-1980's, these assays have also been used in food testing laboratories. The range of detection tests available now include tests for *Listeria*, *Salmonella*, entero-haemorrhagic *E. coli* 0157:H7, aflatoxins, meat species and antibiotic residues. Although simple to use, for consistency, these assays should be carried out using an appropriate machine plate washer and reader.

Most errors occur during the separation stage of free and bound conjugate which can give rise to false results. The use of a plate washer will help to ensure optimum performance.

Similarly, the use of a plate reader will remove any subjectivity on the part of the operator in interpreting the final result and also provide a hard copy of results.

However, like most laboratory equipment, ELISA washers and readers need regular monitoring and maintenance in order to give consistent and reliable results.

Calibration

Regular calibration checks should be carried out on ELISA equipment. This can be achieved by operating the equipment using an uncoated microtitre plate (normally supplied by either the kit or equipment manufacturer).

For ELISA washers the following points should be noted:

- Ensure the microtitre plate is positioned securely on the plate carrier.
- The washer head should be aligned correctly with the wells on the plate.
- The washer head should be level and at the correct height.

If there is an inadequate dispensing or aspiration of wash buffer, the wash and waste bottle should be checked and, if necessary, their tops tightened.

For ELISA readers the following points should be noted:

- Most readers now have a self test facility, either automatic or operator induced. This will normally re-calibrate the machine and check if the electronics and mechanical components are in a serviceable condition.
- The self test should check for light source power supply, filter holder position and position of the plate in the transport carrier.
- The programmes stored in the reader memory should be checked, and the operator should ensure that the correct parameters are retained for each programme.
- A test printout should also be performed as part of the calibration check. NOTE: Thermal printouts may fade and should be photocopied if they are required to be kept for more than one year.

Checks

Before performing an ELISA wash routine the following washing system checks should be made:

- The wash and waste tubing is clear of blockages.
- The correct wash buffer is being used, and it is within its expiry date.
- Tubing and wash head are primed with buffer before use.
- For automatic machines, the correct programme / programme card is used incorporating the correct number of wash cycles.
- The washer dispenser / aspirate heads are functioning properly.

Before reading an ELISA plate, the following plate reader checks should be made:

- The correct programme is being used.
- The correct filter wavelength is being used.
- Sufficient warm up time is allowed for the reader
- The machine is “blanked” before use. Refer to kit manufacturer’s instructions for blanking requirements.
- Areas which can adversely affect results e.g. contamination such as drops of liquid under the well, scratches or fingerprints on the underside of the well, and dust particles.

This equipment should be used on a flat, dry, clean and vibration proof working area. The area should be free from corrosive vapours, smoke and dust.

Tolerance

For ELISA washers the following points should be noted:

- The kit manufacturer’s wash buffer should only be used with distilled or deionised water.

- Only wash buffer, distilled or deionised (purified) water should come into contact - with the wash bottle.
- The microelisa wells should be filled completely to the top, without creating an overflow into adjacent wells.
- Following aspiration, no residual wash fluid should remain in the wells.

For ELISA readers the following points should be noted:

Results are expressed as optical density values, normally ranging from 0.001 to 3.000. Some samples being tested may have a value of greater than 3.000. A reader may report them as “OVER” or “*”.

Servicing and Maintenance

Regular maintenance of ELISA washers and readers is necessary to ensure optimum performance. A routine should be followed, which for washers would be:

Daily maintenance after use

- Change wash buffer for purified water.
- Flush machine through with distilled water.
- Empty waste bottle.
- Release the pressure and vacuum from the bottles by loosening the tops.

Weekly maintenance

- Using the trough at the back of the plate carrier clean the waste part of the machine by flushing through with an appropriate cleaner e.g. 70% isopropyl alcohol. Care should be exercised in the choice of cleaning chemicals used as some may corrode equipment or leave deposits.
- Clean and wash the aspirate probes using the stylet supplied with the washer.
- Check all tubing for damage, bacterial and fungal growth.

The routine for a plate reader should be:

- During and after each use, check and wipe off any spillages, particularly around the plate carrier.
- After use, ensure the dust cover is placed over the reader and (if applicable) the separate printer.
- Check amount of printer/paper roll on a weekly basis.

Regular servicing should be carried out by a qualified engineer. Most equipment manufacturers offer a one year warranty followed by a variety of service contracts.

For most laboratories, routine service of ELISA equipment should be carried out twice a year.

A file containing service visit reports should be kept, and all equipment should be labelled, showing date of service, engineer's initials, and date next service is due.

Documentation

Laboratories using ELISA washers and readers should hold the following documentation:

Operating manual (supplied by the manufacturer).
Service Record Book.
Preventative maintenance record, listing cleaning steps to be performed.
Standard Operating Procedures
Kit package inserts.

References and further reading

Morgan, M. R. A., Smith, C. J. & Williams, P. A. (eds.), (1992). *Food Safety and Quality Assurance Applications of Immunoassay Systems.* Elsevier Science Publishers Ltd., Amsterdam, The Netherlands.

Organon Teknika, (1991). *ELISA Tests, Principles and Practice*. Organon Teknika Ltd.

GUIDELINE No. 12, 9/01, updated 12/03: RECOMMENDED CHECKS AND CALIBRATION CONSIDERATIONS FOR PIPETTES

1. Introduction

1.1 The object of this guideline is to assist users of pipettes, particularly hand held mechanical pipettes, in the correct pipetting techniques, what type and frequency of checks should be conducted on the pipette, and for those who would calibrate pipettes – a guide to the procedure to be followed.

1.2 It is essential for any scientific work that as many things that might adversely effect the outcome of a process or test are controlled or eliminated. As a result, it is essential that volumes dispensed from a pipette are accurately known and are adequate for the process. Calibration and regular conformance checks need to be carried out to provide confidence of this fact.

1.3 This guideline concentrates on the use of mechanical hand held pipettes as this form of instrument is widely used in the industry. It should be noted, however, that the same general principles apply to all pipette types. Each pipette type has its own inherent potential problems that, if not recognised, could cause errors in the work being conducted e.g. cross contamination risks with mechanical pipettes, and difficulty of adequately cleaning glass pipettes.

1.4 This guideline document should be used in conjunction with the pipette manufacturer's instructions and with reference to the following documents:-

- EA-04/10. Accreditation for Microbiological Laboratories (available from www.european-accreditation.org)

- British Standards
  - BS 700: 1982 Pts 1-3 Graduated Pipettes – Specifications.
  - BS 1132: 1987 Specification for Automatic Pipettes.
  - BS 1583: 1986 Specification for One Mark Pipettes.
  - BS 5732: 1985 Specification for Glass Disposable Pasteur Pipettes.
  - BS 6674: 1985 Specification for Disposable Micro Pipettes.
  - BS 6706: 1986 Specification for Disposable Glass Serological Pipettes.
  - BS 6696:1986 Methods for use and testing of capacity of volumetric glassware.
  - BS EN ISO3696: 1995 Water for analytical laboratory use. Specification and test methods.
  - BS EN ISO 8655:2002 Piston-operated volumetric apparatus.

British Standards are available from the British Standards Institution, Customer Services,
389 Chiswick High Road, London W4 4AL, UK

- UKAS Document LAB 15 Traceability: Volumetric Apparatus'
  UKAS documents are available from the United Kingdom Accreditation Service, 21-47 High Street, Feltham, Middlesex TW13 4UN, UK

2. Definitions

a) 'accuracy' -The closeness of agreement between the nominal volume and the mean volume obtained by testing under controlled conditions.

b) 'precision' - The closeness of agreement between the results of successive measurements of the same volume and carried out subject to all the following:

i) the same method of measurement
ii) the same observer
iii) the same measuring instrument
iv) the same location
v) the same conditions of use
vi) repetition over a short period of time

c)'calibration' - The set of operations which establish, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, or values represented by a material measure or reference material, and the corresponding values of a quantity realised by a reference standard.

d)'National Standard' -
A standard recognised by an official national decision to serve, in a country, as the basis for fixing the value of all standards of the quantity concerned.

3. Pipetting Procedure (Mechanical Pipettes)

3.1 It is important to stress that one pipette may seemingly perform differently with:
i) various operators or
ii) the same operator but under various conditions e.g. warmth transmitted by the hand to the mechanical hand pipette during normal use may effect the performance of some pipettes.

It is therefore essential that correct pipetting technique is used to eliminate, as far as possible, errors associated with different operators. Any test or calibration conducted on the pipette will evaluate the pipette + operator + technique.

3.1.1 Forward Mode

The forward pipetting mode should be followed for all calibration and checking procedures. For calibration, distilled water is used. It is recommended that standard forward pipetting is used for all aqueous based samples.

a) Aspiration
1) Hold the pipette vertically.
2) Depress the push button slowly and smoothly to the first positive stop.
3) Immerse the tip into the solution in the centre of the container. The depth to which the tip is immersed in the liquid depends on the model, (refer to pipette instruction manual).
4) Fill the pipette by allowing the plunger to return, in a slow controlled movement, to its original position. (If the button is released too quickly, there is a danger of liquid rapidly entering the tip causing contamination through contact with the end of the pipette barrel / shaft and inclusion of air bubbles).
5) Withdraw the tip from the sample by lifting the pipette vertically.

b) Dispensing
1) Place the end of the tip against the inside wall of the vessel at an angle between 10 and 40 degrees. (If there is a risk of contamination from the side of the vessel, the tip should not touch the side).
2) Press the push button smoothly to the first stop, wait one second and then press the push button to the end stop.

3.1.2 Reverse Mode

Reverse Mode is ideal for samples which are more viscous i.e. blood, plasma.

In reverse mode pipetting, the blow out stroke is used during aspiration. When the liquid is aspirated, an amount of liquid equal to the displaced blow out of air is added. This amount compensates for the liquid that remains as film inside the tip during dispensing.

a) Aspiration
1) Hold the pipette vertically.
2) Depress the push button slowly and smoothly to the second positive stop.
3) Immerse the tip into the solution in the centre of the container. The depth to which the tip is immersed in the liquid depends on the model (refer to pipette instruction manual).
4) Fill the pipette by allowing the plunger to return, in a slow controlled movement, to its original position. (If the button is released too quickly, there is a danger of liquid rapidly entering the tip causing contamination

through contact with the end of the pipette barrel / shaft and inclusion of air bubbles).

5) Withdraw the tip from the sample by lifting the pipette vertically.

b) Dispensing

1) Place the end of the tip against the inside wall of the vessel at an angle between 10 and 40 degrees. (If there is a risk of contamination from the side of the vessel, the tip should not touch the side).

2) Press the push button smoothly to the first stop.

4. Checking Procedures (Mechanical Pipettes)

4.1 It is presumptuous to assume a general frequency for how often a pipette should be checked. Such a frequency would depend on the environment of its use, accuracy requirements, how often the item is used, and how often it needs to be cleaned or dismantled. However, in all cases pipettes should be checked regularly to an accuracy that their use requires.

4.2 Refer to the manufacturer's recommendations for servicing. Replacement of seals, 'O' rings and any other parts may be required e.g. at 6 monthly intervals.

4.3 For conformance checks in between servicing, it is advised that this is based upon the requirements of the standard operating procedure for the application in question. Below are a few suggestions. All results from checks need to be recorded.

Note: the time scales between checks may be extended if it can be shown that the instrument is stable over a set period of time e.g. if consecutive daily checks over a period of time show no change in the performance of the pipette, a change to weekly checks may be justified, and subsequently, weekly checks may be extended to monthly checks etc.

4.3.1 Daily Functional Test

Trouble shooting for leakage's, broken parts and air bubbles; check that the volume taken up in the tip is representative of the volume to be dispensed. A visual check may be sufficient - by using two pipettes to aspirate the same volume. If more precision is required, the use of calibrated balances may be indicated.

This confidence is more noticeably validated using manufacturer's quality guaranteed tips.

4.3.2 Weekly Check

As 4.3.1

4.3.3 Monthly Check

To measure accuracy and a rough estimate of precision, take 4 sample volumes of distilled water at the minimum volume of the pipette. Weigh the samples on a calibrated balance and compare the weights of each dispensed volume (see section 5.5). Repeat the exercise at the maximum volume.

It is important to use a balance, whenever possible, that has a higher degree of resolution than the accuracy required for the pipette. If there is an unsatisfactory spread of results, the pipette may need servicing and re-calibration.

4.3.4 Quarterly Test

To comprehensively evaluate accuracy and repeatability, follow the same procedure as 4.3.3 but conduct the test on 10 sample volumes (see section 5.5).

4.3.5 Performance Test

To test the reliability of an instrument, follow the same procedures as 4.3.3 but test approximately 30 + sample volumes (see section 5.5).

5. Calibration Procedures (normally undertaken by the manufacturer)

The calibration procedure for hand held Piston Operated Volumetric apparatus – Gravimetric method for the volume ranges 20µL - 10,000µL (see BS EN ISO 8655:2002) is used for testing piston and / or plunger operated volumetric apparatus (P.O.V.A.) for errors under prescribed conditions.

5.1 Calibration Equipment

All equipment used to perform calibration tests should have appropriate and current UKAS calibration Certification. Equipment and materials required are as follows:

Equipment	Range / Capacities
Analytical Balance	The balance used shall be dependant on the volumes to be measured e.g. 20µL will require a balance with a full scale deflection of perhaps 20g reading to four decimal places.
Thermohygrometer	0-100% RH, 0-60°C
Thermometer	15-25°C
Water Container	250mL
Distilled water	Grade 3 of BS EN ISO3696:1995

5.2 Calibration Criteria

5.2.1 Test Frequency

Ten repeat measurements to determine both accuracy and repeatability should be performed on all delivery systems. The test should be performed upon introduction into service, following routine and other maintenance, and as the calibration standard operating procedure directs.

Note: the frequency of calibration shall depend on the stability of the instrument. If the instrument is found to be out of tolerance when tested, the calibration period should be reduced to minimise risk (see BS EN ISO8655:2002)

5.2.2 Test Conditions

The P.O.V.A. shall be tested under the following conditions.

Temperature	16°C to 25°C.
Relative humidity	45% minimum

5.2.3 Consumables

This calibration method is applicable only when using P.O.V.A. as per manufacturers recommendations including tips.

5.3 Gravimetric Calibration Method

The principle of the method is as follows.

The general procedure is based upon the accurate determination of the mass of a number of volumes based upon the knowledge of the density of water at specific temperatures and corrections for air buoyancy.

For adjustable mechanical pipettes, four sets of readings should be made: one each at the lowest and highest, and at two more intermediate capacity values, one of which should be below the mid point setting. It may also be of an advantage to conduct a further test at the point of the adjustable pipette's most common usage.

For multi-channel pipettes, readings should be made at the two outside channels and at the centre channel across the range of the pipette capacity as described above.

For the calibration, an appropriately accurate balance is required (see above) and the following should be observed:

i) The pipette should be allowed to stand in the test room for one hour before the test to enable it to reach equilibrium temperature.

ii) If testing adjustable pipettes, the capacity should be set to the required value.
During the weighing cycle, the setting of adjustable pipettes should not be altered.

Iii) Only those tips recommended or supplied by the manufacturer should be used. Where possible the tip should not be touched when inserting it on the tip holder shaft of the pipette.

iv) The same tip should be used for the whole weighing cycle.

5.4 Pipette Specifications

Performance specifications can be confusing. The following is an explanation of the terms, accuracy and precision and how these are calculated.

5.4.1 Imprecision

Imprecision is the quantitative assessment of the random dispersion of a set of replicate measurements such as standard deviation or coefficients of variation.

This information is particularly important whenever a specific term is used to denote a particular type of precision, such as between laboratory, within day or between days. In this document, imprecision refers to a series of groups of data (repeatability) and will be expressed as a standard deviation.

Calculate the standard deviation (S) of the volume delivered from the equation:

$$S = \sqrt{\left(\frac{\Sigma (X - x)^2}{n-1}\right)}$$

Where:

X is the individual sample measurement in mL
x is the mean volume delivered by the handpipette in mL
n is the total number of measurements taken

Compare the result S with the manufacturer's published information.

5.4.2 Inaccuracy

Calculate the error (A) of the handpipette, expressed as a percentage, from the equation:

$$A = \frac{(x-v)}{v} \times 100$$

Where:

v is the nominal volume of the handpipette in mL.
x is the mean volume delivered by the handpipette.

Compare the result A with the manufacturer's published information.

6. Maintenance

6.1 Decontamination.

If there is any chance that a pipette has become contaminated the following procedure is recommended prior to conducting any maintenance however reference to the manufacturer's instructions is recommended - some pipettes can be autoclaved:

Using the appropriate dilution of a viral, bacterial and fungal and sporicidal disinfectant, wipe the pipette over. Badly contaminated pipettes can be soaked in the solution up to the top of the tip holder or if necessary completely immersed.

After soaking for about 20 minutes, the pipette should be rinsed and thoroughly dried.

Note: Pipettes which have been completely immersed will require extended drying times.

6.2 Quick Cleaning

1) Disassemble the pipette
2) Remove the tip holder carefully so that you do not lose any parts. If the tip holder is dirty, soak in the disinfectant. Do not scrape the surface with any sharp objects (the tip holder and the ejector can be autoclaved, if required).
3) To clean the piston simply wipe with lint free cloth, replace seal and 'O' ring with clean hands and with great care.
4) Re-assemble the pipette, taking care to insert the tip ejector fully.
5) Re-check the pipette volumes delivered.

Appendix 1

Example for checking the operation of the Anova and calculations

	Results entered as X.XeX		Log_{10} of the numbers	
	Test sample duplicate results			
	1	2		
sample 1	3.6E+04	2.5E+05	4.5563	5.3979
sample 2	1.8E+04	4.9E+04	4.2553	4.6902
sample 3	2.8E+05	2.5E+05	5.4472	5.3979
sample 4	1.6E+06	2.0E+06	6.2041	6.3010
sample 5	4.8E+03	1.1E+03	3.6812	3.0414
sample 6	1.0E+03	9.7E+02	3.0000	2.9868
sample 7	4.0E+03	4.0E+03	3.6021	3.6021
sample 8	9.0E+06	7.0E+05	6.9542	5.8451
sample 9	3.2E+04	2.0E+04	4.5051	4.3010
sample 10	6.0E+03	3.4E+03	3.7782	3.5315
sample 11	8.0E+05	8.0E+05	5.9031	5.9031
sample 12	2.0E+05	3.5E+05	5.3010	5.5441
sample 13	8.9E+04	3.6E+04	4.9494	4.5563
sample 14	1.5E+05	2.2E+05	5.1761	5.3424
sample 15	1.6E+05	7.5E+04	5.2041	4.8751
sample 16	2.3E+06	2.3E+06	6.3617	6.3617
sample 17	5.4E+04	4.2E+04	4.7324	4.6232
sample 18	3.3E+03	3.5E+03	3.5185	3.5441
sample 19	1.2E+03	1.5E+03	3.0792	3.1761
sample 20	2.3E+05	2.3E+05	5.3617	5.3617
sample 21	8.9E+03	3.6E+03	3.9494	3.5563
sample 22	4.8E+05	1.1E+05	5.6812	5.0414
sample 23	1.8E+03	4.9E+03	3.2553	3.6902
sample 24	1.0E+04	9.7E+03	4.0000	3.9868
sample 25	1.3E+06	1.6E+06	6.1139	6.2041
sample 26	5.2E+03	1.0E+04	3.7160	4.0000
sample 27	5.0E+03	3.0E+03	3.6990	3.4771
sample 28	3.0E+05	3.0E+05	5.4771	5.4771
sample 29	7.3E+03	2.0E+03	3.8633	3.3010
sample 30	4.5E+06	5.5E+06	6.6532	6.7404
sample 31	9.2E+05	1.2E+06	5.9638	6.0792
sample 32	4.0E+03	5.0E+03	3.6021	3.6990
sample 33	7.3E+04	2.0E+04	4.8633	4.3010
sample 34	7.8E+03	7.9E+03	3.8921	3.8976
sample 35	4.5E+03	1.6E+03	3.6532	3.2041

sample 36	4.0E+04	8.0E+04	4.6021	4.9031
sample 37	1.8E+05	1.1E+05	5.2553	5.0414
sample 38	7.3E+03	7.4E+03	3.8633	3.8692
sample 39	8.1E+03	7.1E+03	3.9085	3.8513
sample 40	6.3E+05	5.0E+05	5.7993	5.6990
sample 41	9.2E+02	1.2E+03	2.9638	3.0792
sample 42	4.5E+04	5.0E+04	4.6532	4.6990
sample 43	1.8E+03	1.1E+03	3.2553	3.0414
sample 44	1.5E+03	2.3E+03	3.1761	3.3617
sample 45	1.2E+04	1.5E+04	4.0792	4.1761
sample 46	5.0E+05	1.0E+06	5.6990	6.0000
sample 47	3.0E+05	3.5E+05	5.4771	5.5441
sample 48	5.0E+05	3.0E+05	5.6990	5.4771
sample 49	3.0E+04	3.0E+04	4.4771	4.4771
sample 50	7.3E+05	2.0E+05	5.8633	5.3010
sample 51	9.0E+05	4.5E+05	5.9542	5.6532
sample 52	4.5E+03	5.5E+03	3.6532	3.7404
sample 53	4.5E+04	1.6E+04	4.6532	4.2041
sample 54	9.0E+05	1.0E+06	5.9542	6.0000
sample 55	6.2E+03	5.0E+03	3.792	3.699
sample 56	4.0E+05	8.0E+05	5.602	5.903
sample 57	7.8E+04	7.9E+04	4.892	4.898
sample 58	1.8E+04	1.1E+04	4.255	4.041
sample 59	8.0E+03	7.0E+03	3.903	3.845
sample 60	1.0E+06	8.0E+06	6.000	6.903
sample 61	7.4E+03	6.3E+03	3.869	3.799
sample 62	1.5E+03	2.3E+03	3.176	3.362

Anova: Single Factor

SUMMARY

Groups	Counts	Sum	Average	Variance
Row 1	2	9.954242	4.977121	0.354176
Row 2	2	8.945468	4.472734	0.094579
Row 3	2	10.84509	5.422549	0.001211
Row 4	2	12.50514	6.252574	0.004695
Row 5	2	6.722633	3.361316	0.204703
Row 6	2	5.986771	2.993385	8.75-05E
Row 7	2	7.204119	3.602059	0
Row 8	2	12.79934	6.399670	0.615100
Row 9	2	8.806179	4.403089	0.020832
Row 10	2	5.309630	2.654815	0.030423
Row 11	2	11.80617	5.903089	0
Row 12	2	10.84509	5.422549	0.029533
Row 13	2	5.505692	2.752846	0.077258
Row 14	2	10.51851	5.259256	0.013833
Row 15	2	10.07918	5.039590	0.054139
Row 16	2	12.72345	6.361727	0
Row 17	2	9.355643	4.677821	0.005956
Row 18	2	7.062581	3.531290	0.000327
Row 19	2	6.255272	3.127636	0.004695
Row 20	2	10.72345	5.361727	0
Row 21	2	7.505692	3.752846	0.077258
Row 22	2	10.72263	5.361316	0.204703
Row 23	2	6.945468	3.472734	0.094579
Row 24	2	7.986771	3.993385	8.75E-05
Row 25	2	12.31806	6.159031	0.004065
Row 26	2	7.716003	3.858001	0.040327
Row 27	2	7.176091	3.588045	0.024608
Row 28	2	10.95424	5.477121	0
Row 29	2	7.164352	3.582176	0.158086
Row 30	2	13.39357	6.696787	0.003797
Row 31	2	12.04296	6.021484	0.006657
Row 32	2	7.301029	3.650514	0.004695
Row 33	2	9.164352	4.582176	0.158086
Row 34	2	7.789721	3.894860	1.53E-05
Row 35	2	6.857332	3.428666	0.100842
Row 36	2	5.505149	2.752574	0.045309
Row 37	2	10.29666	5.148332	0.022872
Row 38	2	5.732554	2.866277	1.75E-05
Row 39	2	7.759743	3.879871	0.001637
Row 40	2	11.49831	5.749155	0.005037
Row 41	2	6.042969	3.021484	0.006657

Row 42	2	9.352182	4.676091	0.001046
Row 43	2	4.296665	2.148332	0.022872
Row 44	2	6.537819	3.268909	0.017230
Row 45	2	4.255272	2.127636	0.004695
Row 46	2	11.69897	5.849485	0.045309
Row 47	2	11.02118	5.510594	0.002240
Row 48	2	11.17609	5.588045	0.024608
Row 49	2	8.954242	4.477121	0
Row 50	2	11.16435	5.582176	0.158086
Row 51	2	11.60745	5.803727	0.045309
Row 52	2	7.3936	3.6968	0.003801
Row 53	2	4.8573	2.42865	0.100845
Row 54	2	11.9542	5.9771	0.001048
Row 55	2	5.4914	2.7457	0.004361
Row 56	2	11.5052	5.7526	0.045300
Row 57	2	9.7897	4.89485	1.51E-05
Row 58	2	8.2967	4.14835	0.022876
Row 59	2	7.7482	3.8741	0.001682
Row 60	2	12.9031	6.45155	0.407794
Row 61	2	7.6685	3.83425	0.002443
Row 62	2	4.5378	2.2689	0.017223

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	196.5565	61	3.222238	58.76377	1.4E-38	1.525532
Within Groups	3.399692	62	0.054833			
Total	199.9562	123				

Square root of Within Groups MS is calculated using
=sqrt(0.054834)

= 0.23 This is the Repeatability Standard Deviation.

2 x Repeatability Standard Deviation ≈ 95% confidence limit

= ± 0.47 This is the practical approach to 'uncertainty value'

This is just under 0.5 of a log

