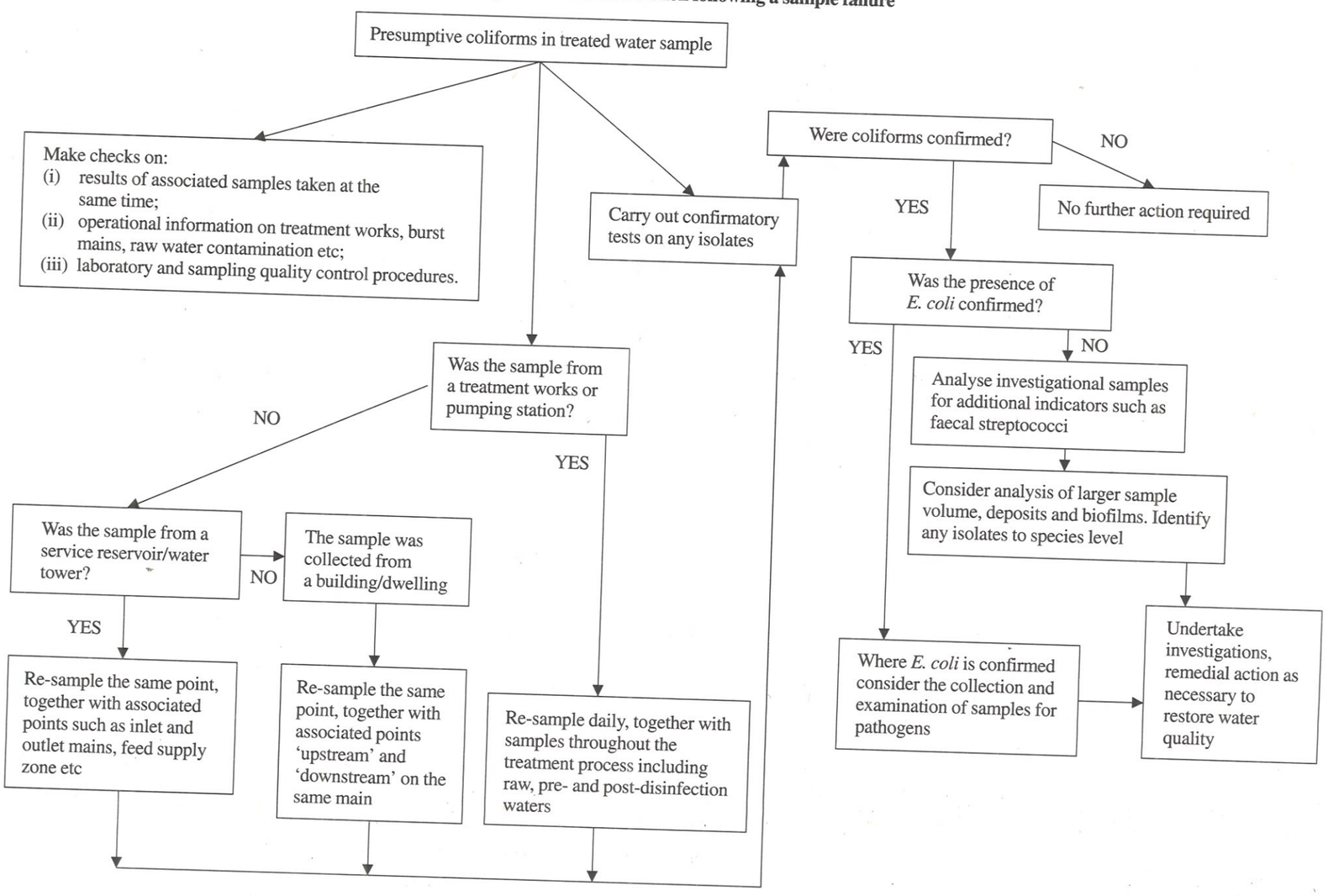
Investigative samples from all these points should be collected daily and at different t on each day until it has been demonstrated that coliforms are not present in the w passing into supply. Where coliforms are isolated from water entering the distribu system, distribution samples should be taken to determine the extent of any dissemin contamination. The performance of the plant must always be regarded as inadequate it can be demonstrated unequivocally that either; (i) the sampling facility is representative of the water in supply; (ii) the sample tap does not comply with requirements given in section 6.4.6.1; (iii) the samples were in a batch which fa laboratory quality control procedures; or (iv) the source of the contamination of the w or fault with the treatment process has been remedied. Whilst water free from colifo may be achieved by immediate remedial measures such as increased dosing with chlo or removal of a given source in the case of (iv), the efficacy of the water treatm processes should always be re-evaluated. This can be achieved by the collection analysis of large volume samples of both raw and treated water which should be exami for secondary bacterial indicators such as faecal streptococci and other more resist organisms such as enteroviruses, *Giardia* and *Cryptosporidium*.

These procedures are outlined in Figure 1, which is not intended to be exhaustive.

Where any failure is regarded as giving rise to, or is likely to give rise to a significant ri to health of persons residing in the area, the Drinking Water Inspectorate, Local Author and District Health Authority must be informed. Prior consultations between Lo Authorities, District Health Authorities and water undertakers may be necessary establish what constitutes a significant risk.

Figure 1 Outline of action following a sample failure



Chapter 5: Special Considerations

5.1 Water in Food Production

Water supplied for use in food production falls within the scope of the Regulations. A time of publication of this report regulations under the Food Act are being prepare MAFF. Legislation will control the deterioration in water quality after the time of su which may affect the wholesomeness of the foodstuff in its finished form.

5.2 Hospital Water Supplies

Hospitals are usually served by public water supplies, but several have their own pri (usually borehole-derived) supply. Some use public and private supplies, someti blended. Most will have one or more storage tanks to balance fluctuating demand enable a supply to be maintained for a limited period in an emergency. Many hosp complexes consist of a variety of buildings of different ages with pipework constructe different materials. There are often long and complicated pipe runs, sometimes w deadlegs. There have been reports of microbiological contamination of cold water syste where storage tank covers have been allowed to deteriorate or were absent (Thomas, Pi and Maurer 1972, Tabaqchali, Stevens and Gazides 1977, Anon 1978). Poorly construc systems may contain inappropriate materials or allow temperature rises in cold suppl with consequential bacterial growth. Water softening devices associated with the co water supply may become colonised with bacteria (Anon 1979). Mycobacteria have be found in hospital hot and cold water systems (Collins, Grange and Yates 1984).

As well as water of potable quality for drinking, washing and general hygienic purpose considerable quantities are required for catering. The hospital's population will inclu patients of extremes of age and of varying degrees of immune competence, and so may b more vulnerable to water-associated infections. Potable quality water is required for pr operative hand washing and high speed drills used in dentistry. Water for renal dialysi pharmaceutical preparations and for some purposes in pathology laboratories will requir additional treatment such as reverse osmosis, ion-exchange or distillation. It is therefo important to ensure a supply of good quality water at all times. General guidance on th installation of a cold water supply is given in the DHSS Health Technical Memorandum 2 (DHSS/WO 1976, currently under revision) which contains details of suitable filtratio and disinfection processes. The Code of Practice on The Control of Legionellae in Healt Care Premises (DH/WO 1988) also includes a section on the design, operation an maintenance of hot and cold water services systems.

Water used for drinking, washing and culinary purposes must comply with the require ments of the relevant regulations whether derived from public or private supplies. Routine microbiological monitoring of a hospital's private supply will therefore be required by its local authority for the parameters specified in the Private Supplies Regulations.

For hospitals with their own private supply, the regular examination of samples at source is recommended in order to detect any variation in quality that may require an adjustment to any treatment carried out. It is also strongly recommended that, regardless of the type of supply, regular checks of the microbiological quality of the water during distribution should be made.

If a sample is found to contain coliforms, or if the colony count is significantly higher than the usual level, immediate investigation is required. At the least, further samples from the same and other points served by that part of the supply should be examined. The hospital's Infection Control Doctor (ICD) must be notified immediately of any such occurrence. Close and continuous liaison between the hospital estates department and the ICD should in any case be an established feature of hospital management. There should be a routine maintenance programme including regular inspection of taps, shower heads, storage tanks and other installed devices.

If major problems arise, the CCDC should be advised and assistance sought from the local environmental health department and the nearest public health laboratory. The CDSC may also provide assistance. If there is major contamination, it may be necessary to boil the water to be used for drinking, food preparation etc or to obtain an emergency tanker or bowser supply. The use of bowsers should be carefully supervised to ensure that the contents do not become contaminated—in any event the water quality should not be taken for granted and all bowser water used for drinking or cooking should be boiled before use.

5.3 Trains, Aircraft, Ships, Bowsers etc

Where water of potable quality is required in circumstances that prevent it from being supplied directly from a mains piped supply, special care will be required to ensure that it is wholesome and remains so throughout the duration of the supply. This applies particularly to potable water supplies in trains, long-distance coaches, aircraft and ships where the supply may have to be maintained over considerable periods from storage tanks.

All storage tanks, bowsers etc that are used for potable supplies must not be used for any other purpose and must be so constructed that they can be completely drained and emptied. Materials of construction should not be capable of supporting microbiological growth, and must be capable of withstanding chlorination to a level of 50 mgL^{-1}. The connecting pipework should be similarly constructed. Bowsers and tankers should be fitted with lockable lids and taps to prevent contamination. All such water storage facilities must be cleaned and disinfected by chlorination before being filled with water of potable quality. They should be regularly drained, cleaned and disinfected at appropriate intervals. Care must be taken during the filling process to ensure that contamination, for example of nozzles, does not occur. Staff engaged in handling these water supplies should maintain a high standard of personal hygiene and not be required to perform other duties that might involve the handling of contaminated materials (such as from toilets etc). They should also be subject to regular health surveillance. Tanks should be drained and cleaned if necessary after each period of use.

The microbiological quality of water supplied by such means should comply with the standards in the Regulations. 'In use' samples should be tested at appropriate intervals to ensure that quality is maintained. As the necessary measures taken to ensure the delivery of water of potable quality will depend upon the nature of the vehicle concerned, further guidance is detailed below.

5.3.1 Trains

Storage tanks on railway buffet cars should be filled with water from a piped main via a standpipe and hose, or from a bowser filled from a standpipe. The standpipes and hoses should be protected from contamination and flushed before filling the bowser or storage tank. The tanks and bowsers should be drained, cleaned if necessary and disinfected at regular frequent intervals. Hose nozzles should be kept immersed in a suitable disinfectant solution when not in use. Where an ultra-violet treatment unit is fitted in the pipe system between the storage tank and the point of use, it must be regularly maintained. Details of the necessary procedures are given in the report of a joint British Rail/Institution of Environmental Health Officers working party on the supply of water for train catering (BR/IEHO 1985). Appropriate procedures should also be applicable to the supply of wholesome water on long-distance coaches.

5.3.2 Aircraft

In general, supply arrangements are similar to those outlined for trains. However, the source water may not always be of potable quality and it may be necessary to chlorinate the water before use. Hypochlorite solution or a solid chlorine-donor sufficient to maintain a residual chlorine concentration of 0.2 mgL^{-1} should be used. More detailed information can be found in the 'Guide to Hygiene and Sanitation in Aviation' (WHO 1977).

5.3.3 Ships

The supply system must be used exclusively for the distribution of potable water. Care must be taken to maintain the integrity of the system and to avoid the possibility of contamination. Cross-connections with non-potable water systems and back flow must be avoided. Water taken on board should be of potable quality and care must be taken to

ensure that it is not contaminated during loading; it is likely to require addit chlorination to ensure it remains wholesome. On-board chlorination plants mus adequately maintained. Ultra-violet treatment systems should not be relied upon a sole means of disinfection. Reverse osmosis and evaporator units should not be unless the vessel is sufficiently far from land to ensure that the inlet water is not subje pollution. Detailed recommendations for freshwater storage and distribution on shi given in the Department of Transport Notice M.1214 (DT 1986).

5.3.4 Tankers and bowsers

All tankers and bowsers used for the delivery of potable water must be dedicated for t purpose and suitably identified. They should be kept locked and drained when not be used and disinfected and flushed with potable quality water before use. Care must be ta to avoid contamination during use and the quality of the contained water cannot be assu for longer than 24 hours. Water from tankers and bowsers should be boiled before be used for drinking purposes.

5.3.5 Water in containers

The Directive also applies to potable waters sold in bottles or other sealed containers. T quality of water from private supplies should be monitored prior to bottling under t provisions of the Private Supplies Regulations. The quality of water from public suppli is controlled by the Regulations. MAFF has issued regulations which came into force on April 1994 and which set quality standards for water in containers after the point of supp (SI 1994/743, see Appendix E). The MAFF regulations will not control natural mine waters which are already covered by separate legislation, or waters which are, or ha been, issued with a product licence under the Medicines Act 1968.

5.4 Drinks Vending Machines

5.4.1 Machine types

Drinks vending machines may be hand filled or permanently connected to a water supp system.

5.4.2 Sampling

5.4.2.1 Hand filled machines

These machines will be filled from a convenient cold water supply tap using a suitabl vessel (for example, a jug). When sampling, take samples from the cup station and th water storage tank.

5.4.2.2 'Plumbed-in' machines

Most plumbed-in drinks vending machines do not have the facility for dumping water to waste and when these machines are used at the start of each day they are likely to draw water which has been standing in the supply pipework overnight. **It is therefore inappropriate** to sample the water entering the machine after running water to waste.

The sampling procedure for plumbed-in machines should be based upon the following sequence;

(i) take a sample from the supply pipe entering the machine **without** running any water to waste; and

(ii) take a vended sample from the cup station of the machine.

For further details and additional guidance see the recommended methods for sampling water supplied to and from machines in Drinks Vending Machines—Code of Practice on Hygiene and Water Supply (AVAB 1987).

5.4.3 Interpretation of results

Standards recommended to its members by the Automatic Vending Association of Britain (AVAB 1989) include the following;

(i) *E. coli* and *Pseudomonas aeruginosa* shall not be present in any 100 mL sample of water dispensed by the machine;

(ii) presumptive coliform organisms should not be present; if they are found in the water supply to the machine this should be investigated further;

(iii) the total viable (colony) counts shall be less than 10000 colony forming units per mL at 22°C and 1000 colony forming units per mL at 37°C in water dispensed by the vending machine, and normally not more than ten times greater than the number in the supply water entering the machine (not flushed to waste).

Chapter 6: Methods and Procedures

6.1 Safety Within the Laboratory

The Health and Safety at Work etc Act 1974 and regulations relating to the Contr Substances Hazardous to Health (COSHH) place a clear duty on management with re to safety in laboratories to ensure, so far as is reasonably practicable, the health, safety welfare at work of all employees. Similarly, employers are enjoined not to expose health and safety of the public to risk.

Implementation of this Act is not solely a management responsibility, for safety contin to rest with heads of departments, although they may delegate certain duties. Equa individual employees have a duty to co-operate with their employers and to t reasonable care for the health and safety, not only of themselves but also of their fell workers and of the general public. In order to ensure that the requirements of the He and Safety Act are being met, the Health and Safety Executive may inspect the premis The Health and Safety Inspectorate also has the power to close laboratories and, extreme instances, to prosecute.

Sound technique is the basis of safe microbiological procedures. It is important that individuals concerned with this work should receive adequate training, and that t training is recorded. It is also important that the necessary laboratory equipment a facilities should conform to accepted codes of safety and good practice (Collins, Hartl and Pilsworth 1974, NWC 1983, HSAC 1991a, 1991b, PHLS 1993).

It is accepted that there is no control over the organisms or agents that may be in wat samples received in the laboratory. A distinction is therefore made between the occasion isolation and the deliberate introduction of certain pathogenic organisms to the laborator

6.1.1 Classification of micro-organisms, based on risk of infection

Micro-organisms have been categorised into four groups (1–4) in increasing order o hazard to laboratory workers or the community (ACDP 1990). The great majority o organisms handled in the water microbiology laboratory will fall into Hazard Groups 1 o 2. On rare occasions an organism may be isolated that falls into Group 3 (for exampl *Salmonella typhi*). In such an event all the cultures should be removed to a Containmen Level 3 laboratory and any further work continued there.

6.1.2 Basic laboratory (Containment Levels 1 and 2)

Laboratories of this type are intended for work with organisms that fall into Hazard Groups 1 and 2. Ceilings, walls and floors should be non-absorbent, smooth, easy to clean and disinfect, and resistant to the chemicals that will be used in the area. Floors should be non-slip and lighting and heating adequate. A hand basin, other than a laboratory sink, is essential. Benching should be set at a height that is comfortable to work at when seated. Benching should be smooth, resistant to the chemicals used and easy to clean and disinfect.

6.1.3 Containment Level 3 laboratory

Laboratories of this type are intended for work with organisms that fall into Hazard Group 3. They should contain all the features outlined for the basic laboratory but in addition have the following features.

The Level 3 laboratory should be separate from other laboratories, with no access (for example false ceilings, pipe ducts) other than through the entrance door, which should be lockable, and an air transfer grille which is normally fitted to the door. A Type 1

microbiological safety cabinet should be sited in the laboratory away from direct draught of doors and windows which could interfere with its proper function.

A lower pressure in relation to the other laboratories should be maintained in the containment laboratory so that air movement is only one way. All air from this room should be released to atmosphere with no re-circulation to other areas within the laboratory building. During working hours this can be achieved by extraction of air from the room to atmosphere through the safety cabinet with replacement air entering through the door grille.

An international biohazard warning sign should be attached to the door and access should be strictly regulated.

6.1.4 Good practice within the laboratory

Micro-organisms can be ingested by mouth pipetting, from articles that may be contaminated if taken into the laboratory, for example pencils, food, cigarettes and cosmetics, and contaminated hands. Micro-organisms can be taken into the lung by inhalation of bacterial aerosols produced during many routine laboratory operations, for example pipetting, centrifuging, blending. Entry through the skin may result from accidental stabbing with pipettes, contaminated broken glassware or used hypodermic needles and through contamination of cuts and abrasions. Eyes splashed with contaminated material can also lead to infection.

It is therefore important that mouth pipetting is prohibited under all circumstances and that pipetting devices are provided in the laboratory.

Food and drink must never be taken into, stored or consumed in the laboratory. Pipettes, pens, labels etc must never be put in the mouth.

Use of hypodermic needles and glass Pasteur pipettes should be avoided wherever possible and chipped or cracked glass vessels replaced. Glass homogenisers should be wrapped in a cloth and gloves worn.

All equipment should be regularly inspected and any faults and remedial actions noted.

Centrifuge tubes should not be filled to more than two-thirds of their capacity. Work surfaces should be disinfected regularly (usually at the end of the working day) and always after a spill of infectious, or potentially infectious, material. A suitable disinfectant, ready for use should always be available at each work station together with a discard jar for re-usable pipettes and other small items, which should be fully immersed. Discard jars should be emptied frequently and the disinfectant replaced.

Spilt infectious or potentially infectious material and broken culture vessels should be covered with absorbent disposable cloths and soaked in disinfectant, left at least 30 minutes and then cleared into a suitable container.

Properly supported discard bags or bins should be available at each work station, including one that is appropriately robust to contain 'sharps'. No discarded infectious or potentially infectious material should leave the laboratory until it has been sterilized or placed into properly labelled bags for incineration.

All materials that fall into Hazard Group 3 should be processed in a microbiological class 1 safety cabinet sited in a Containment Level 3 laboratory.

Scratches, cuts and abrasions on exposed parts of the body should be covered with waterproof dressings and hands should always be washed after handling infectious material and when leaving the laboratory, after removal of laboratory coats.

Proper protective clothing should be provided and worn correctly fastened at all times when in the laboratory. It should be removed when leaving the laboratory and stored apart from other clothing. It must never be worn in any other areas such as rest rooms and canteens. Separate protective clothing must be provided for use in the containment laboratory.

Further details on containment levels, use of microbiological cabinets and cate
of pathogens according to hazard are given by the Advisory Committee on
Pathogens (ACDP 1990).

6.2 Quality Assurance

It is essential that a laboratory is able to demonstrate that all results emanating f
fit for the purpose for which they are to be used. This is achieved by implem
appropriate programme of quality assurance. The Regulations define fitness fo
ance purposes for the monitoring of drinking water supplies as "capable of esta
within acceptable limits of deviation and detection, whether the sample contains
trations or values which contravene the prescribed concentrations or values". De
on the particular test being used, it is necessary to be able to demonstrate the pres
absence) of a particular class of micro-organisms in a given sample volume
estimate numbers. The detection of small numbers of organisms is particularly im
Because of the ubiquitous nature of most microbes, it is essential to ensure t
organisms that are detected have originated from the original sample and have n
introduced inadvertently during sampling or analysis.

Any effective quality assurance programme must therefore cover the whole proces
sample collection to the interpretation and reporting of results. It will also include a s
of internal quality control, and participation in a proficiency testing scheme, also re
to as external quality control or assessment, appropriate to water microbiology.

Internal quality control should include procedures for and records of the con
monitoring of working practices, equipment and reagents. These procedures s
include the use of blanks, semi-quantitative positive controls, negative controls, refe
materials, replicate analyses of samples and control charts.

6.2.1 Definitions

ISO 8402–1986, Quality—Vocabulary, contains the following definitions:

Quality assurance: All those planned and systematic actions necessary to provide adequ
confidence that a product or service will satisfy given requirements for quality.

Quality control: The operational techniques and activities that are used to fulfil requi
ments for quality.

The International Harmonised Protocol for the Proficiency Testing of (Chemical) Anal
tical Laboratories (IUPAC/ISO/AOAC) contains the following definitions:

Proficiency testing scheme: The system of objectively checking laboratory results by
external agency. It includes comparison of a laboratory's results at intervals with those
other laboratories, the main object being the establishment of trueness. Proficiency testi
is designed to assess accuracy.

Internal quality control: The set of procedures undertaken by the laboratory staff fo
continuous monitoring of operations and results in order to decide whether the results ar
reliable enough to be released; internal quality control primarily monitors that batchwis
trueness of results on quality control materials, and precision on replicate analysis of tes
materials.

Quality assurance programme/system: The sum total of a laboratory's activities aimed at
achieving the required standard of analysis. While internal quality control and proficiency
testing are very important components a quality assurance programme must also include
staff training, administrative procedures, management structure etc. Accreditation bodies
judge laboratories on the basis of their quality assurance programme.

6.2.2 General requirements

Laboratory facilities should be constructed from materials that are robust and easily
cleaned. There should be adequate space for dealing with anticipated workloads, with
separate areas for the reception of samples, examination of samples, preparation of media
and reagents, sterilization and wash-up, and storage (including refrigeration). The

laboratory layout should provide a smooth workflow. Benching should provide an adequate and safe linear space for each worker, of which not more than half should be occupied by equipment. Facilities for the examination of samples under special containment may be required when pathogens are being handled. Adequate ventilation and lighting must be provided throughout the laboratory areas.

Microbiological tests must be carried out by suitably trained and qualified personnel in accordance with approved methods. All methods of analysis, including instructions for the preparation and sterilization of reagents and media, must be documented and up to date copies readily available to the analyst. All subsamples, results of culture and other tests, and test reports must be clearly linked with the original sample at all stages of analysis. Staff must be instructed in the safe use of equipment and handling of micro-organisms and the safe disposal of cultures. A laboratory safety manual should be available for easy reference and there should also be a clearly defined system for reporting accidents. The staff should be so organised that, at any one time, each member has a particular area of responsibility and is under the supervision of a single person who is responsible for the overall management of the laboratory. Due regard should also be given to security and confidentiality.

6.2.3 Management

Every laboratory should have an organisational chart showing every member of staff, their roles in day to day performance of tasks and, importantly, the chain of accountability and reporting. The post responsible for the Quality Assurance programme should be clearly defined and each member of staff should have a well-defined job description outlining responsibilities and their limits. A record of staff training should be maintained and regularly updated. There should also be a defined plan for provision of cover for work when staff members are absent.

The reporting system should enable results that require immediate remedial action to be communicated without delay to appropriate persons. Records of laboratory results should be kept for as long as necessary to comply with requirements for archive and audit trails, and should include the date, place, and time of sampling.

The Quality Assurance programme can only be successful if all constituent parts are documented. However, it will provide a valuable source document covering all activities of the laboratory and should only require periodic reviews thereafter.

6.2.4 Laboratory equipment

All microbiological laboratories will require certain basic items of equipment including incubators, water baths, centrifuges, refrigerators and freezers, microscopes, autoclaves, balances, thermometers, pH meters and water purifying apparatus. Such equipment will need regular inspection, cleaning and maintenance and, where appropriate, checking for accuracy.

6.2.4.1 Thermometers

The accuracy of thermometers or other temperature-measuring instruments will require periodic checking against certified reference thermometers. Thermometers should be capable of recording differences of 0.1°C. Ideally, waterbaths and incubators should be provided with continuously recording thermometric instruments. Electronic temperature recording devices are now available that can be used continuously to monitor temperatures inside equipment, and data stored can be reviewed and inspected.

6.2.4.2 pH meters

pH meters will require regular standardisation and should be calibrated before use, using standard buffer solutions. Buffer solutions with pH values in appropriate ranges are available commercially.

6.2.4.3 *Balances*

Balances should be provided with a range of sensitivity that is appropriate for the am of substance to be weighed. Balances should be kept clean, serviced periodically and calibration checked and be traceable to national standards.

6.2.4.4 *Pipettes*

Pipettes may be glass (re-usable) or plastic (disposable); all re-usable and a represent sample from each batch of disposable pipettes should be calibrated and be traceab national standards. Calibrated automatic pipettes may also be used. See also Append

6.2.4.5 *Water purifying equipment*

A de-ioniser will provide water of satisfactory chemical quality for microbiolog purposes. The life of the unit may be prolonged by using distilled water or water that been produced by reverse osmosis. Containers and pipework should conform to BS 6 (BSI 1988). The water quality will require regular monitoring with a conductivity me and to ensure absence of contaminating bacteria the water should be passed throug suitable filter before use. Filters must be renewed at regular intervals.

6.2.4.6 *Sterilizing equipment*

(i) Autoclaves

Autoclaves may be used to sterilize items of laboratory equipment, reagents, media etc a also to render infectious materials safe for disposal. It is important to ensure that t appropriate temperatures and times are attained in all parts of the load. Autoclaves shou be checked periodically to ensure that the required time/temperature relationships a attained and each operating cycle should have a chart record on which the cycles a clearly identified. They must be used in accordance with the appropriate safety requir ments, particularly when the load consists of fluids in sealed containers. A Britis Standard, BS 2646 Parts 1, 3 and 5 (BSI 1993), gives guidance on the use and performanc requirements for laboratory autoclaves.

(ii) Hot air ovens

These also require regular monitoring of the time/temperature relationships for eac operating cycle. They should be fitted with a fan to ensure an even distribution of heat t all parts of the load, see BS 3421 (BSI 1961).

Other forms of sterilizers should be validated before use.

6.2.4.7 *Refrigerators and cold rooms*

Refrigerators should have their operating temperatures (normally between 1 and 5°C) checked regularly and be de-frosted at regular intervals. Cold rooms should be equipped with a continuous recording device. Stored items should be inspected periodically and discarded at the end of their storage life.

6.2.4.8 *Freezers*

These also require regular temperature checks to ensure that they comply with the manufacturers' specifications and should be de-frosted at appropriate intervals. They could be fitted with over-temperature alarms. Their contents will need to be periodically inspected and unwanted items discarded.

6.2.4.9 *Incubators and water baths*

The temperature control of incubators, thermal blocks and water baths is of particular importance in water microbiology. Frequent checks should be carried out to ensure that the temperature does not vary by more than ± 0.5°C when in use. When testing coliforms for thermotolerance at 44°C the temperature should not vary by more than ± 0.25°C. Fill water baths with deionised water and check the water level regularly; clean as necessary. Different loading patterns of incubators will give rise to different temperature attainment

patterns in parts of the incubator. This is particularly important in 44°C incubators and standard loading patterns should be documented, validated and adhered to.

6.2.4.10 Membrane filter holders

These require checking for leaks and sterilizing before use and cleaning after each use.

6.2.4.11 Microscopes

The optics and stage should be cleaned with lens tissue paper after each use. UV microscopes require radiation intensity to be checked, and all microscopes should be serviced, at regular intervals.

6.2.4.12 Glassware, reagents etc

All glassware items such as pipettes, flasks, Petri dishes etc used in the preparation of media or the handling of samples will need to be of suitable quality, free from inhibitory substances and adequately cleaned and sterilized before use; in many instances pre-sterilized plastic alternatives can be used. The accuracy of volumetric equipment should be traceable to national standards. Water used in the preparation of media and reagents has to be of high quality, for example de-mineralised or distilled. Chemicals and other substances used in the preparation of reagents and media will also need to be of appropriate analytical quality.

6.2.5 Internal quality control

To ensure that a laboratory is capable of isolating, accurately identifying and enumerating micro-organisms present in a sample, and to avoid contamination during this process with extraneous micro-organisms, it is necessary to have a system of internal quality control. This constitutes submitting to the usual isolation, enumeration and identification procedures samples known to contain micro-organisms similar to those normally sought as well as samples that are sterile. Control tests need to be carried out on all the isolation, enumeration and identification procedures and includes all confirmatory tests. If the procedures are functioning satisfactorily, such micro-organisms will be detected, or in the case of the sterile sample no micro-organisms will be found. The control procedures should be done in parallel with each batch of samples examined.

Control organisms should be freeze-dried wild-type reference strains, obtainable from the National Collection of Type Cultures (NCTC), and reconstituted and diluted with quarter strength Ringer's solution to contain a suitable number. The use of natural water known to contain relevant organisms may also be suitable. All confirmatory tests should include positive and negative controls.

Prepared media should be checked for sterility, and identified by batch reference number. A sample from each batch should be tested to ensure that it will support the growth of appropriate organisms. If selective, it should be checked for the inhibition of growth of unwanted organisms. All media will need to be stored under suitable conditions that will delay deterioration (usually in the dark, and refrigerated if necessary) and discarded at the expiry of their shelf life.

Where membrane filters are used, they should be of suitable quality and tested in the chosen media to ensure that they will support the growth of organisms being sought.

If automatic counting instruments are used these should be tested and calibrated against known values.

6.2.6 Control charts

Quality control charts are used extensively in water chemistry for control of laboratory procedures. It is possible to extend the use of quality control charts to microbiology. However, the natural random variation in the number of organisms found between sub-samples effectively means that wide control limits are necessary. In addition, there may be problems in distinguishing between the chance discrepancies in the number of organisms

present in sub-samples and in determining these numbers, ie the true laborato
Methodology is being continually developed, for example on the use of split sa
check that processing of a counting method, such as, for example membrane filtr
being performed consistently. Additional samples, examined in duplicate,
included with each batch of routine samples to provide within-batch quality contr
duplicate samples can be considered as two halves of a single sample, and the
plotted on a graph on which allowable limits of variation are included. Often, actio
are set at certain values to trigger investigation of the method.

Because of the random nature of the distribution of micro-organisms in water, exami
of two halves of the same sample can result in quite wide variation in the counts obt
For example, if the count reported for the first half of a (duplicate) sample is 5, the
95% confidence interval (CI) for the count for the second half will be 0–14. The CI f
count in the second half-sample for those counts observed in the first half-sample ra
between 0 to 100 are given in Table 13 in Appendix C.

Water microbiology laboratories should carry out several duplicate analyses regularly
plot the results on a control chart. The two parts of each sample should be treate
separate samples and put through the system in a routine manner. The samples shoul
positioned at random in the incubator, and these positions changed frequently w
different batches of samples are examined. The results should, if possible, be read bl
(ie, the count from the second half-sample should be made in ignorance of the result fr
the first half-sample), and should be read by the same person.

The first count should be recorded on a control sheet, and the corresponding CI for
second (paired) count entered from Table 13. The second half-sample count is th
recorded alongside these figures. If this count falls outside the CI, the fact should
highlighted. The results can also be plotted to give a sequential, visual check on a 'cont
chart'. If, over a period of time, the second count falls outside the CI on more than 5%
occasions, investigations should be carried out to determine the cause. (Lightfoot et
1994).

6.2.7 External quality control

Laboratories should participate in a system of external quality control which involves th
examination of samples distributed by an external agency. Such samples should be
examined as part of the usual laboratory routine. The laboratory's results can then be
compared with those obtained by other participating laboratories and so provide an
independent check on the quality of the laboratory's performance over time.

6.2.8 Laboratory accreditation

Various schemes are in operation whereby laboratories may be accredited to an approved
standard. In addition, the performance of laboratories carrying out analysis for compliance
purposes under the Regulations in England and Wales is now subject to scrutiny by the
Drinking Water Inspectorate. An agreement has been reached between DWI and the
NAMAS Executive on all aspects of drinking water compliance sampling and analysis.
Requirements are defined in the Drinking Water Testing Specification. Accreditation of a
laboratory to this specification will normally be regarded by the Inspectorate as demon-
strating compliance with the relevant regulations.

6.3 Statistical Considerations

A bacterial count from a sample yields valuable information, but it is important to assess
the accuracy of this information. There are two main aspects to be considered. First, what
can be inferred from this sample about the level of bacterial contamination in the water
source from which the sample was taken? Secondly, how accurate was the count for this
sample using the method chosen by the laboratory? These two sources of 'sample error'
are often confused and this can lead to mis-understandings in the interpretation of a sample
count. Generally, it is the first source of sample error which is the more important (Tillett
1993). This potential inaccuracy should be understood both by microbiologists and by
those submitting samples for analysis since it affects the interpretation of results and any
subsequent actions. The second source of sample error should be brought to the attention
of laboratory staff who control the analytical procedures used.

The expression 'bacterial count' will be used to imply the count of organisms being enumerated, for example the total coliform count. The same principles apply to the counting of viruses or other organisms.

6.3.1 Estimating bacterial counts within the water source

The examination of a single sample gives an indication of the bacterial count at a particular point in the catchment or supply and at a particular time. The place at which a sample is collected will have been carefully chosen (see section 6.4) and thus a sample should be typical of the sampling area. The volume of water sampled may not however possess identical characteristics to adjacent volumes of water with respect to microbiological quality. Indeed, only a very small volume of water is examined in the laboratory compared with the body of water in question.

The CI for a bacterial density in a body of water cannot be estimated from a single sample. A mathematical description of the distribution of bacteria is needed before ranges, such as 95% confidence intervals, can be estimated. The only situation where a single sample will give such an estimate is when the bacteria are distributed at random. In this case the appropriate mathematical description is the Poisson formula which has a single parameter (μ = mean = variance) which can be estimated, albeit very approximately, from the single sample count. However, there is no evidence that relevant bacteria are ever randomly distributed in any part of a water system.

For untreated waters, there tends to be enormous variation in microbiological quality (Tillett 1993). In treated waters contamination may be intermittent and organisms may be present in aggregates, often on particulate matter, rather than evenly dispersed. Samples from the same sampling point closely adjacent in time can show large differences in bacterial count.

The parameters describing the distribution of bacteria may change over time and therefore a series of single samples collected at different times cannot be used to estimate confidence intervals for the bacterial contents of the body of water at any one time. They should be used instead to indicate trends over time.

6.3.1.1 Monitoring for trends

Bacterial counts from a series of routine single samples collected from one sampling point at different times should be plotted graphically and studied for trends. With untreated waters, any sustained rises above the usual seasonal range should be investigated. An explanation should also be sought for any single, grossly abnormal count. There is no satisfactory mathematical definition of 'abnormal'. If a plot of routine results is monitored visually, excessively high counts should be obvious, as should changes in the average count, over and above any seasonal variation. With treated waters, the organisms should be absent and any positive findings of indicator organisms should be investigated as described in sections 4.6.2.2 and 4.6.3. Low levels of contamination or erratic contamination will produce some samples which do not yield indicator organisms. The microbiological examination of the water must be seen as only part of the total surveillance procedures. Again, it is impossible to estimate the likelihood of the presence of organisms in the body of water simply by using results from one routine sample.

The sampling of treated waters should be as frequent as practicable in order to increase the chance of detecting any breakdown in microbiological quality of the water and these frequencies are specified in the Regulations. The required sampling frequencies are based on past experience which has aimed at maintaining generally high quality of water at the point of delivery. It would be impractical to sample at such a frequency and with such speed as to prevent all water-borne microbiological disease, but past practice in the UK has kept the numbers of such outbreaks relatively low (Galbraith et al 1987, Benton et al 1989). Constant re-appraisal in the light of new experience (DoE/DH 1990) should aim to reduce the chance of such outbreaks.

6.3.2 Estimating the accuracy of counting bacteria from a single sample

Inaccuracies can arise from inadequate sampling procedures and from prolonged dela unsuitable storage conditions between collecting the sample and examining it i laboratory. These will not be considered here, neither will accidental contaminatio

Two sources of possible inaccuracy which will be considered are the effects of dilution the method of counting organisms, both of which come into the category of the sec source of error (inherent in laboratory methods) discussed in the first paragraph of sec 6.3.

6.3.2.1 Potential imprecision due to dilution of the sample

Samples of treated water should not need to be diluted before examination. Samples heavily contaminated untreated water may have to be diluted so that a count can be ma If the multiple tube method is used then some tubes, but not all, should show growth. If t membrane filtration method is used then the membrane or plate should not be overgrow

It is standard procedure to report bacterial counts of indicator organisms as the number organisms per 100 mL. With undiluted waters, approximately 100 mL of sample a examined (105 mL in the case of the 11-tube series). If the sample has to be diluted (pric to any additional dilution inherent in the multiple tube method) and this dilution is, say fo example, 10-fold then only approximately 10 mL of the original sample will be examined The count obtained is then multiplied by the dilution factor and the new count per 100 mL is now an estimate of the numbers contained in the 100 mL sample. The original 100 mL will have been thoroughly agitated to try to achieve random distribution of the organisms Therefore the count from the diluted portion can be used to estimate the likely count for the original 100 mL and confidence intervals can be calculated using random distribution theory. The methodology for doing this has been published (Tillett and Farrington 1991) and some examples are shown in Table 2.

The imprecision introduced by dilution is likely to be relatively small compared with the variability in bacterial density in a water source where counts are high enough to require dilution of the sample before examination. It is suggested that the confidence intervals given in these examples in Table 2 are not stated when the results are reported. Quoting such ranges may be mis-understood and taken as a statement about the likely bacterial density in the water source. Table 2 is meant to illustrate the additional imprecision introduced when volumes smaller than 100 mL are actually examined.

Table 2 Estimated count per 100 mL (EC) and 95% confidence intervals (CI) for number of organisms in a 100 mL sample where only a subsample is examined, following dilution.

Organisms observed in the subsample:	10-fold dilution		100-fold dilution	
	EC	CI	EC	CI
10	100	50– 180	1000	480– 1830
50	500	380– 650	5000	3750– 6640
100	1000	820–1200	10000	8190–12200

6.3.2.2 Other imprecision of laboratory methods

Samples of treated waters should contain zero indicator organisms and the presence of very small numbers of such organisms in a sample can be detected and measured with good precision by the methods recommended in this Report. Published work has shown (Tillett 1986) that the multiple tube method is more sensitive than the membrane filtration technique. However, untreated waters may yield moderate or high bacterial counts and in these situations the accuracy with which the count is made by the chosen laboratory method should be considered.

6.3.2.3 *Multiple tube method*

In this method a series of subsamples is taken from the original sample, and processed to ascertain which subsamples show the presence of one or more relevant organisms. A mathematical formula based on laws of probability can be used to estimate the most probable number (MPN) of organisms present, given the number of tubes in the series which show growth (McCrady 1915, 1918, Cochran 1950). These various mathematical approaches have been reviewed by Eisenhart and Wilson (1943), and Cochran (1950) has described the principles involved in the estimation of bacterial densities by dilution methods. Tables have been developed by Swaroop (1938, 1951) which give a greater number of combinations of positive and negative results, some of which in practice should occur only very rarely (Woodward 1957, Man 1975).

A confidence interval was often published with the MPN which demonstrated the uncertainty of the bacterial count for that water sample, as estimated from multiple tubes, not for the water source, although it has often been confused with the latter. For this reason the use of a confidence interval is not recommended.

In the past, the relevant mathematical equations had to be solved approximately. However, the power of modern computers allows exact solution of the probability of counts associated with each dilution-series result (Tillett and Coleman 1985, Tillett 1987). Whilst the re-calculation of most probable numbers shows little discrepancy with previously published values, these new calculations have thrown light on two issues. First, the imprecision of previously published confidence intervals. Second, for moderate or high bacterial density, the multiple tube method does not give a clear 'most probable number'. There is a 'most probable range' (MPR) of counts, all of which are almost equally likely to be as correct as the MPN. All calculations are based on the assumption that the organisms present in the water are evenly distributed and the importance of thorough mixing of the sample cannot be stressed too strongly. Although the multiple tube method is very sensitive for the detection of a small number of indicator organisms, the MPN is not a precise value. Apparent differences between results must therefore be interpreted with caution. It should be appreciated also that variations in bacterial numbers in the water source may be very much greater than any imprecision introduced by the multiple tube method.

Appendix C gives tables of the MPN and the appropriate MPR for a 6-tube series (1×50 mL; 5×10 mL), an 11-tube series (1×50 mL; 5×10 mL; 5×1 mL) and for a 15-tube series (5×10 mL; 5×1 mL; 5×0.1 mL). Results should be reported with care and it may be appropriate to quote the MPR rather than the MPN in order to illustrate the imprecision of the method. However, it should be clearly stated that the range applies to the sample and not to the water source. When plotting results for trends it will be more practical to use the MPN.

6.3.2.4 *Membrane filtration method*

Counts on membranes are subject to statistical variation and replicate tests on the same sample are unlikely to give the same number of colonies. If 100 mL of sample is filtered and incubated, and then every relevant colony on the membrane counted, and every colony confirmed, then the presumptive and confirmed counts are as precise as the method allows. No statistical imprecision need be considered. If the sample is diluted prior to filtration then the count becomes an estimate of the density in the undiluted sample, as already described. If the presumptive count is taken from only a segment of the membrane then imprecision is introduced comparable to that introduced by diluting the sample, assuming that the segment is typical of the whole plate.

6.3.3 Confirmation

Confirmatory tests of the presumptive colonies present on a membrane should be carried out. Two situations are common which, when there are multiple colonies present, can lead to different approaches being adopted when deciding how many colonies should be tested for confirmation. If the aim is to estimate the count of the relevant colonies then consideration should be given to the imprecision introduced by confirming only a fraction of the number of colonies present, and the examples given below should be studied. The number of colonies tested (for confirmation) should be chosen to give a sufficient level of

accuracy. This may require subculturing all colonies on a membrane with fewer than presumptive colonies present.

If the aim is to demonstrate the presence or absence of the organism, then a differ approach may be chosen, provided that there are no microbiological contra-indicatio The presence of the organism is demonstrated as soon as óne positive confirmation made. A laboratory may therefore choose to examine fewer colonies, initially, than w the aim is to estimate the count. However, if the (few) colonies chosen show negative, th the sample cannot be assumed to be satisfactory since other colonies not chosen confirmatory testing may, if tested, prove positive. Hence other colonies from the sto membrane should be tested. This sequential testing is acceptable only when refrigerat storage of the membrane is not detrimental to the survival of the relevant organism.

If **all** presumptive colonies are tested to confirm their nature then no further imprecisio (other than that due to the test method) is introduced when the presumptive count converted into a confirmed count. If only some of the colonies are tested by confirmato methods then further imprecision is introduced into the confirmed count. For example, if presumptive count is made by counting **all** the typical colonies on a plate (to be referred as N) then it is common practice to make confirmatory tests on some, but not all, of thes colonies, unless N is very small. Let n be the number of colonies tested and let x be th number which are confirmed to be of the relevant organism type. The confirmed colon count is then estimated to be xN/n. For example, if 60 colonies were observed on the plate and 20 were selected at random for testing, and 15 of these were confirmed, then the estimated confirmed count would be $15 \times 60 / 20 = 45$.

It is assumed that the n colonies are selected at random or by some other procedure which ensures that they are a typical subsample of the N colonies and that all N colonies were equally likely to be from the relevant organism group. The conditional probability that y is the true count, given that x colonies have confirmed, can be calculated from an equation of possible combinations:

$$P(x \mid y) = {}^{y}C_{x} \,.\, {}^{N-y}C_{n-x} \,/\, {}^{N}C_{n}$$

The 95% CI for the confirmed count can be found by studying these probabilities for all possible values of y, using the observed value of x. The CI will exclude end of range high and low values of y such that their cumulative conditional probabilities sum to less than or equal to 0.05. This procedure follows the principle described in full by Tillett and Farrington (1991).

As an example, if 10 colonies are observed (N) and only two are tested (ie n=2) then Table 3 shows the complete range of probabilities.

Table 3 Range of probabilities if 10 colonies are counted and only two are tested for confirmation

Given that y =	the probability that		
	x = 0 is	x = 1 is	x = 2 is
0	1.000}	–	–
1	0.800}	0.200}	–
2	0.622}	0.356}	0.022
3	0.467}	0.467}	0.067}
4	0.333}	0.533}	0.133}
5	0.222}	0.556}	0.222}
6	0.133}	0.533}	0.333}
7	0.067}	0.467}	0.467}
8	0.022	0.356}	0.622}
9	–	0.200}	0.800}
10	–	–	1.000}

The brackets (}) show the range of y values which should be included in the 95% CI for the true count. Note that if one of the two colonies tested confirms (x = 1) then all the possible values of y are within the 95% CI, because all the probabilities exceed 0.05.

In general, if only a small number of the total colonies is tested then the CI can be very wide. They also tend to be wider if a substantial number of colonies turn out not to confirm. Some examples are shown in Table 4.

Table 4 Variation in the 95% CI with variation in proportion of tested colonies confirmed

Colonies observed (presumptive count)	Number tested	Number confirmed	Confirmed count	95% CI
10	2	0	0	0– 7
10	2	1	5	1– 9
10	2	2	10	3–10
14	7	5	10	6–12
50	10	5	25	9–41

With treated waters, where the vast majority will yield no or very few presumptive colonies, it is recommended that all colonies should be tested by confirmatory methods to ensure accuracy. For untreated waters it may be worth considering using the presumptive count rather than introduce the additional sampling error which accompanies confirmation of some and not all of the colonies. It should be noted that the practice of confirming a maximum of 10 colonies can still introduce potential imprecision of several fold, especially if the presumptive count is large and some colonies fail to confirm.

6.3.4 Comparing results with recommended standards

The standards for drinking waters concentrate on the presence or absence of indicator organisms and pathogens. No statutory comparisons need be made with actual counts and therefore the potential problem of how to compare actual (for example membrane filtration) and estimated (for example multiple tube) results need not be addressed with respect to potable waters.

6.3.5 Reporting results

The report should be a clear statement of the findings. A further statement on sample error, to qualify these findings, should NOT be necessary for routine samples. The sampling strategy should have been worked out with the aim of acquiring an adequate level of information.

If it is decided that a report for a special sample warrants some statement on accuracy then a clear distinction should be made between sampling error due to variability at the water source and possible error introduced by the laboratory methods.

Absence of organisms and unmeasurably high counts should be reported as follows:

(i) **No organisms detected.** A water sample in which no relevant organisms are detected should be reported as 'none found in the sample examined'. There is no correspondence with the concept of 'limit of detection' which is used with chemical measurements, and therefore an expression such as '< 1 per unit volume' has no meaning.

(ii) **Overgrowth, or all tubes positive.** This means that the laboratory method has failed to estimate the true count because of insufficient pre-dilution. With the multiple tube method it is customary to report this as '> 180' for the 11 tube series or '> 1800' for the 15 tube series, in the appropriate units. In fact, the count could be very much higher than these lower limits. With membrane filtration and other methods the report should be 'count too high to be estimated at the dilution used'.

6.4 Sampling Procedures

6.4.1 Sample containers

Sample bottles should be made from good quality soda or borosilicate glass, or from a suitable pre-sterilized disposable or autoclavable polymer (plastic), and be free from toxic substances. They should be provided by the laboratory and used exclusively for the purpose of microbiological sampling. Glass bottles should not be permitted for sampling

on food manufacturing premises or in recreational pool areas, where alternative ste plastic bottles should be provided. The size of the bottle depends upon the number type of tests to be carried out. A capacity of about 300 mL should be sufficient and bot should be fitted with ground glass stoppers or autoclavable or heat-resistant screw caps silicone rubber liners which will withstand repeated sterilization at 160°C. As alternative ground glass stoppers can be used in conjunction with disposable strips prevent jamming. Single-use pre-sterilized bottles can also be used. Glass bottles must capable of withstanding the conditions required for sterilization. Any containers show defects should be discarded.

6.4.2 Container preparation

New re-usable bottles and caps should be washed thoroughly with a phosphate-free, no toxic detergent followed by thorough rinsing with clean and distilled or deionised wat and drained. Used bottles should be rinsed in distilled water. This procedure can be carri out either manually or in a washing machine.

As the water to be examined is likely to contain chlorine or chloramines, sufficient sodiu thiosulphate should be added to sample bottles to neutralize these substances. At p values normally occurring in a water supply, sodium thiosulphate ($Na_2S_2O_3.5H_2O$) at concentration of 18 mgL^{-1} should neutralize up to 5 mgL^{-1} of free and combined residu chlorine and has no significant effect on the coliform or *E. coli* contents of unchlorinate water on storage (PHLS 1953). It is therefore recommended that at least this level o thiosulphate should be added to all microbiological sampling bottles before they ar sterilized, the amount depending on the size of the bottle. Thus 0.1 mL of a 1.8% m/ solution of sodium thiosulphate should be added *pro rata* for each 100 mL of bottl capacity. The sodium thiosulphate should be prepared freshly and added by means of dispenser used only for that purpose. Bottles should then be capped and where necessary the cap covered with another suitable additional cover, before sterilization. Periodically after sterilization, a check can if necessary be carried out to ensure the sodium thiosulphate has not been de-activated.

If bottles with ground glass stoppers are used, a strip of paper or foil, for example approximately 75 × 10 mm should be inserted between the stopper and the neck of the bottle before sterilization. This prevents jamming of the stopper and cracking of the glass on cooling. Before sterilization, bottles should be marked with suitable indicator tape capable of showing that the bottle has been through the sterilization process.

Bottles should be sterilized, for example either by autoclaving at 121°C to 122°C for 20 minutes or by heating to 160°C in a fan-assisted oven and holding at that temperature for 60 minutes. Each batch of bottles sterilized should contain a suitable indicator, for example Browne's tube and thermax strips or thermalogs for autoclaves and Browne's tubes and thermax strips for ovens, to show that it has received appropriate heat treatment. The indicators should be placed at appropriate levels within the load being sterilized. The results obtained for all indicators should be checked and recorded. Bottles should only be released for sampling if the sterilizing process is shown to be satisfactory. Sterilizing equipment should be serviced and properly calibrated in terms of time and temperature performance, (see section 6.2.4.6). Sterile bottles should be dated and should be stored in a location provided only for that purpose. A quality system of stock control should be carried out to ensure sterilization and that expiry records are maintained. Sample bottles should be stored in such a way that they should be used within two months of sterilization. Any bottles beyond that expiry date should be washed and re-sterilized as described above. Pre-sterilized tamper-proof bottles bought as such should be used within the shelf-life specified by the manufacturer.

6.4.3 Container labels

6.4.3.1 Pre-scheduled samples

All sample bottles must be adequately labelled. Self-adhesive labels which are easily removed on washing are suitable for this purpose. Labels may be pre-printed or hand written in indelible ink. The information provided should clearly identify the sample and