MANUAL LABORATORY FOR THE MICROBIOLOGICAL ANALYSIS OF DRINKING WATER

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Manual laboratory for the microbiological analysis of drinking water

- sterilization
- sample collection
- microbiological media and storage
- laboratory facilities

Sterilization

- Sterilization is the killing or removal of all microorganisms, including bacterial spores which are highly resistant.
- Sterilization is an absolute term, i.e. the article must be sterile meaning the absence of all microorganisms.
- Sterilization in Microbiological works like preparation of culture media, reagents and equipments where a sterile condition is to be maintained.

CLASSIFICATION OF METHODS

Sterilization and disinfection are done by :

(A). Physical Agents

1. Heat
2. Radiation
3. Filtration

(B). Chemical Agents

- In practice, certain methods are placed under sterilization which in fact do not fulfill the definition of sterilization such as boiling for 1/2 h and pasteurization which will not kill spores.

STERILIZATION BY HEAT

- The most common method used for elimination of microorganisms

- reliably effects
- easy of use
- economic
- destroy all microorganisms and their
- steam under 1 atm of pressure, at 121

15min. of exposure in autoclaves

Application:

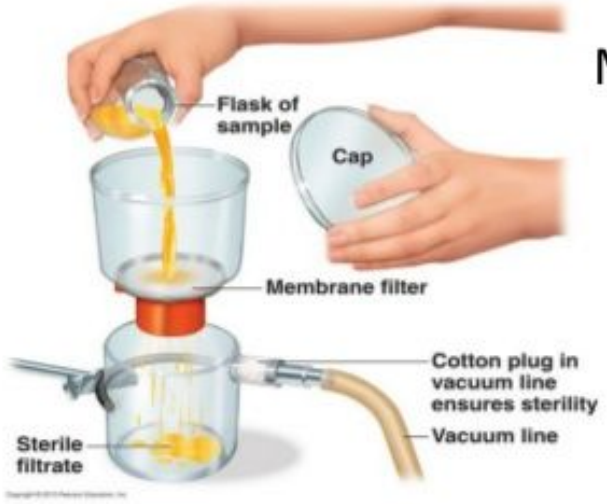
- Glassware and other dry materials—15 min.
- Contaminated and discarded materials—30 min

Dry Heat

- Hot Air Oven - It Is one of the most common method used for sterilization. Glass wares, swab sticks, all-glass syringes, powder and oily substances are sterilized in hot air oven.
- For sterilization, a temperature of 160°C is maintained (holding) for one hour. Spores are killed at this temperature. It leads to sterilization.

Sterilization: physical methods

Filtration for liquid not tolerant to heat, rays, chemical agents.



Membrane filters present pores with diameters of µm or nm to effectively remove microorganisms.

Filtration sterilization

- Filtration process does not destroy but removes the mocroorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non viable particles.
- The major mechanisms of filtration are sieving, adsorbtion, and trapping within the matrix of the filter material.

  e.g. HEPA filters

STERILIZATION AND DISINFECTION
Chemical agents

- Alcohols
- Aldehydes
- Halogens
- Phenols
- Surfactants
- Heavy metals
- Dyes
- Gases (ethylene oxide, oxidants)

Ultraviolet radiation

- The 254 nm range is used for germicidal purposes
- Must penetrate to surface to be effective
- Vegetative microorganisms are more susceptible than bacter

STERILIZATION CONTROL

- to ensure that potentially infectious agents are destroyed by adequate sterilisation regimes
- three levels:

-physical: measuring device control (temp., time, pressure)

- chemical:

• substances that undergo a colour change or have melting points within the sterilizing range

STERILIZATION CONTROL

- biological:

•Bacillus stearothermophilusspores(104-106organisms)
-survives steam heat at 121ºC for 5 min. and is killed at 121ºC in 13 min.
-validate and determine the adequacy of steam or chemical vapor sterilisation
•Bacillus subtilisspores
-validate and determine the adequacy of ethylene oxide or dry heat sterilisation

Sample collection

- Sampling for microbiological analysis is performed in accordance with EN ISO 19458 – Water quality – Sampling for microbiological analysis
- General information in respect to the sampling from distinct water bodies is given in the respective parts of ISO 5667.

Sample collection – Sampling points

- Water quality monitoring objectives are:
  - Water quality assessment provided by producers and distributors of water
  - Water quality at the consumer's tap

Samples must be taken from representative points.

Sample collection – Sampling points

- Sampling points where conditions are unstable should be avoided , and the heterogeneity of the hydraulic system shall be taken into consideration
- In studies on the efficacy of disinfection, the sampling points shall be chosen to ensure that the reaction is complete

Sample collection – Sampling technique

- Personnel
- Sample containers
- Filling procedure

Sampling technique

1. Personnel

- Sampling must be ensured by specially designated staff for this activity.
- Regular training of personnel, trainings and establishing jurisdiction for the registration of all who sampled water must be well documented in the laboratory or institution.

Sampling technique

2. **Sample containers**

- Four routine samples, use clean, sterile bottles.
- The volume of the bottles should be adequate four analysis of all requested parameters.
- Bottles can be made of glass or various plastics. Usually glass is preferred for re-use, and polyethylene is used as disposable.
- Bottle openings closed with plastic or glass stopper should be further protected from contamination by, e.g.: aluminium foil.
- Autoclave bottles at $(121\pm3)^0$C for at least 15min.
- If necessary, sterilize bottles in a dry oven for at least 1 h at $(170\pm10)^0$C

Sampling technique

Inactivation of desinfectants

- To assess the microbiological quality of water desinfected by an oxidant (e.g. chlorine, chloramine, bromine or ozone), stop the action of the oxidant as soon as the sample is taken. Add a reducing agent such as sodium thiosulfate pentahydrate ($Na_2S_2O_3$ X $5H_2O$) = 18 mg/ml) to the sample bottles.

- Thus, 0,1 ml of sodium thiosulfate pentahydrate solution is added for each 100ml of bottle capacity.

Sampling technique

3. Filling procedure

Potable water from a tap

Sampling at a tap can have different purposes:

a) to determine the quality of the water in the distribution main (which is the responsibility of the distributor);

- sample to assess the quality in the main are best taken at special taps that are close to the main distribution, clean, without attachments and disinfectable by flaming or suitable equivalent
- normal taps may be used to assess the quality in the main, if they are disinfectable by flaming but in case of unclear results, consider the service network as potential source of contamination.

Sampling technique

3. Filling procedure

b) to know the quality of the water at is flows from the tap to be consumed

- In this case, tap disinfectable by flaming are not always available and other disinfection methods (application of hypochlorite solution, ethanol or isopropanol) need to be considered.

c) to know the quality of the water as it is consumed, i.e. as it flows out of the (possibly contaminated) tap.

- is the method to assess the quality of drinking water in special situations, e.g. outbreaks.

In this case, do not: remove attached devices and inserts, disinfect the tap, flush.

Sampling technique

Sampling form

Uniquely identify and label the bottle, and fill in the sampling form before, or just after, sampling.

The form shall at least indicate:

- name and address of the client;
- the list of parameters to analys;
- date, time and location of sampling as well as the name of the person who is taking the sample.

Transport and storage

- Keep the time between sampling and analysis in the laboratory as short as possible.
- For drinking waters, analysis should ideally be started within the same working day.
- Cool samples – ideally $(5\pm3)^{0}C$ – during transport (e.g. by using ice packs or melting ice). Take care not to freeze them. Protect samples from sunlight.
- Maximum time between sampling and analysis is 8 h.

Microbiological media and storage

- Chemicals used in a microbiology laboratory should be of analytical grade quality.
- Reagents and chemicals should be stored and used in accordance with manufacturer's instructions and discarded if the expiry date, the date by which the reagent should be used, has passed.

Microbiological media and storage

- Manufacturers can supply most microbiological media as dehydrated formulated preparations.
- Media that are supplied as dehydrated powders should be stored in a cool dry place, and containers labelled clearly with the date of receipt, and the dates when containers are opened.
- Media are prepared by weighing out the appropriate amount of material and adding distilled, deionised or similar grade water

Microbiological media and storage

Preparation of media

- The appropriate weight of the dehydrated formulated preparation, or ingredients as listed in the method, is added to the appropriate volume of water. Whilst it is often not essential to have to adjust the pH of the medium, in certain circumstances it will be necessary to do so.
- Adjusting the pH should be carried out by the addition of small amounts of an appropriate concentration of hydrochloric acid or sodium hydroxide solution until the required pH value is achieved. This is often carried out before sterilisation takes place
- All dehydrated media should be completely dissolved before being dispensed and sterilised.

Microbiological media and storage

Preparation of media

- All dehydrated media should be completely dissolved before being dispensed and sterilised.
- Typically, media are sterilised by autoclaving at 115 °C for 10 minutes or 121 °C for 15 minutes.
- Before a medium is poured into Petri dishes or tubes, it should be cooled, for example in a water bath or incubator, to approximately 50 °C prior to the addition of any supplements.
- When the medium has solidified, the Petri dish should be inverted and stored at a temperature of between 2 - 8 °C in such a way as to prevent excessive drying
- When a medium has been prepared it should be labelled with its batch number and expiry date.

Microbiological media and storage

Quality control of laboratory media

- When the sterilised medium has been poured into Petri dishes, a representative number should be checked to ensure that they are satisfactory.
- These Petri dishes should be incubated at a temperature and time appropriate for the medium and examined for any contaminant microbial growth. Where liquid media are prepared bottles or tubes should be similarly checked.
- Media should also be checked to establish that they support the growth of the target organisms for which they have been prepared, and differentiate or are selective against non-target organisms.
- Appropriate organisms should be identified and tested with each medium

Laboratory facilities

Laboratory Space

- Laboratory space must be adequate to accommodate periods of peak work loads.
- Working space requirements must include sufficient bench top area for analytical equipment, processing samples, storage space for media, glassware and other laboratory items.
- Facilities must be clean, air conditioned, heated (65-80 F), and with adequate lighting at bench top. Humidity levels must not be excessive. Rugs in laboratories are unacceptable. Bench counter tops must be in good condition.
- Outside windows must be covered with appropriate sun blocks.

Laboratory facilities

Laboratory Space

- The laboratory shall provide safeguards to avoid electric shock, prevent fire and accidental chemical spills, and minimize microbiological dangers, facility deficiencies, and equipment failures.
- Work space must be increased proportionally for laboratories engaged in multiple disciplines

Laboratory facilities

Environmental monitoring

- Because of the ubiquitous nature of most of the microbes of interest, it is essential to ensure that any organisms that are detected have originated from the original sample and have not been introduced inadvertently during sampling or subsequent analysis.
- Laboratories should consider appropriate monitoring of the environment, both related to the sampling procedure and the analysis within the laboratory, to ensure that any micro-organisms detected do not adversely contribute to any result.

Laboratory facilities

The laboratory shall:
- have the necessary resources to perform accurate analyzes,
- have procedures for checking that the environment does not affect the analysis,
- to control, monitor and record environmental conditions,
- stop analyzes whether environmental conditions can invalidate results
- separate areas that can contaminate each other, to control access to premises examination provide housekeeping

Access to the laboratory is covered and controlled to ensure confidentiality and security to ongoing work results analysis

Thank you for attention