

*Fact sheet*

**Autoclaves – steam inactivation**

**Dr. V. Küng, P. Thalmann (Küng-Biotech + environment)**

**for the Chemical and Biological Safety department (KCB)  
of the State Laboratory of the Canton Basel City (publisher and copyright)**

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## Fact sheet on autoclaves - steam inactivation

### 1. Principle

All parts of the inactivation material are exposed to the effect of **saturated, confined water vapour** at a specific **temperature** for a specific **time**. The air present in the inactivation chamber and in the inactivation material is removed by displacement or evacuation before the start of the exposure period.

Removal of the microorganisms during the inactivation process follows a first-order reaction (logarithmic removal).

### 2. Scope

Apart from the combustion method, inactivation with steam is the most effective way of inactivating solid and liquid waste or contaminated materials and working equipment. It is the method of choice if inactivation is to take place on the spot and heat and moisture do not pose a problem<sup>1</sup>.

- ? **Exceptions**
- . animal carcasses<sup>2</sup>
  - . furniture, equipment that is sensitive to heat and moisture
  - . spills
  - . radioactivity
  - . certain chemicals

In these cases, chemical or other inactivation methods are to be preferred.

### 3. Definitions

**Exposure time/  
residence time**      During the exposure time, all parts of the inactivation material have assumed the prescribed temperature and are exposed to the action of saturated, confined water vapour.

### 4. Requirements concerning the inactivation process

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<sup>1</sup> The method of choice for inactivating solid and liquid waste is steam inactivation. Methods other than steam inactivation should only be chosen if the latter is unfeasible or unsuitable, e.g. where equipment, facilities and furniture cannot be steam-treated. In these cases, gasification with ethylene oxide or formaldehyde is appropriate (EN 12740). Besides steam inactivation, chemical inactivation also plays a part in the treatment of waste. This is particularly the case if micro-organisms are further processed following harvesting and inactivation in order to isolate certain heat-labile constituents. Waste in the narrower sense of the term is thus not involved here. In selecting the inactivation method, planned further treatment is involved in this case, besides inactivation of the micro-organisms. Other inactivation methods that do not play a role, or only a secondary role, with regard to the issues dealt with here are only briefly touched on in what follows.

<sup>2</sup> Animal carcasses can be autoclaved prior to burning. Autoclaving as the sole inactivation stage is not to be recommended as full steam penetration of the carcase is difficult to demonstrate and is generally not practicable with larger animals.

<sup>1</sup> The method of choice for inactivating solid and liquid waste is steam inactivation. Methods other than steam inactivation should only be chosen if the latter is unfeasible or unsuitable, e.g. where equipment, facilities and **Containers** Containers in which waste is present must not be sealed **in an airtight manner** during treatment in the inactivation chamber. Only use containers with sufficiently large openings or bags. The material to be disinfected must not contain **any hermetically sealed vessels** unless they contain water or aqueous solutions. The quantity of liquid per vessel must be capable of being heated to the inactivation temperature. If **bags** are used as containers, they must be designed so that – if they are sealed – they tear during the first vacuum phase.

**Compensation time** The compensation time and the cooling time are to be tailored to the type of waste. Compact components and the volume of liquid in particular are to be taken into account in this context.

**Effectiveness** Effectiveness must be demonstrated by a test. For example, in accordance with the guidelines for “Testing of waste disinfection methods for effectiveness”, Public Health Office Gazette 36 (1993), pp. 158-160.

? The commonest cause of faults in autoclaves is the inclusion of air in the inactivation material. The nominal values for inactivation are not achieved in these inclusions.

## 5. Recommended values and standard procedures

**Steam, temperature and exposure time** are crucial to inactivation. The following applies as a standard procedure: 15 – 20 minutes, at 121°C and 2 bar pressure. *Bacillus stearothermophilus* spores, which are among the most heat-resistant of microorganisms, are destroyed under these conditions. Free DNA is also destroyed under these conditions.

**Exception** Prions must be autoclaved at 132°C for 1 hour (flow method); 132°C for 18 minutes is sufficient in an autoclave with pre-vacuumising.

On ecological grounds, it may be advisable to treat waste by methods that depart from the standard procedure. However, the effectiveness of different methods of this kind must be shown. In the case of mixtures of organisms, the inactivation conditions are to be tailored to the heat-resistant microorganisms.

## 6. Requirements concerning autoclave operation

- Regulation of **responsibility** (e.g. list of people responsible for regular operation, contacts in the case of malfunctions, person responsible for performing periodic functional checks)
- A **collection point** for the waste to be autoclaved must be organised.
- **Operating instructions** must be posted up in a conspicuous location near the autoclave.

- **Operating instructions** must contain information on the parameters to be observed (temp., pressure, time)
- **The loading plan** (content, number, volume, mass) must be readily visible.
- It is recommended to keep a **log book** for every autoclave (list of “runs”).
- **Indicators** must be positioned at the most unfavourable location for vapour penetration (e.g. greatest volume).

## 7. Types of equipment

A list of tested and approved types of equipment is published by the Robert Koch Institute in Germany<sup>3</sup>. It should be borne in mind that the temperatures and exposure times in the list apply for a limited exposure range and therefore depart from the standard procedure. Generally speaking, an automatic list of operating parameters (residence time, temperature, any pressure) is needed to check the functional sequences. Electronic recording with printout is the state-of-the-art technology; older models are equipped with a so-called recording disk.

### Vacuum method

#### Characteristics

- . Removal of air from autoclave by evacuation for several times alternating with allowing vapour to flow in.
- . Effectiveness depends on the negative pressure achieved and the mode of operation (single or multiple evacuation following supply of vapour).
- . Exhaust air and condensate-forming method.
- . Inactivation chamber and directly connected pipework and valves are leakproof under vacuum.
- . Method usable for biosafety levels 1-4, state-of-the-art technology for 3-4, absolutely vital for 4.

#### Equipment

- . Venting of the autoclave via sterile filter from safety stage 3 of the CO absolutely vital; from safety stage 2, start-of-the-art technology.
- . Controlled autoclaving of the condensate during the inactivation process. Separate inactivation of the condensate is to be avoided.

### Flow method

#### Characteristics

- . Air is displaced by saturated steam.
- . Vapour is produced internally or externally.
- . Bleed valve is closed after several minutes (where appropriate, manually).
- . Forms exhaust air and condensate.

#### Equipment

- . **Steam generator externally**, suitable for safety stages 1-3, venting from safety stage 3 via sterile filter absolutely vital.
- . Condensate outlet must be small, capable of controlled sterilisation from safety stage 3
- . **Steam generator internally**. Suitable for safety stage 1, not state-of-the-art technology in the case of safety stage 2.

## 8. Effluent sterilisation

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<sup>3</sup> A similar list of tested or approved types of equipment for inactivation does not exist in Switzerland.

Liquid waste and effluent from the laboratory and production can be sterilised via continuous or batch methods (discontinuous). Reliability and operational safety for batch methods is considerably greater than for continuous methods (inactivation in the flow method). On safety grounds, it should be borne in mind at the plant construction stage that dead volumes are kept as small as possible (span for the drain valve less than about 5 cm). The design of the inactivation systems necessitates observance of the twin-barrier principle: the autoclave represents the first barrier, while a downstream uncontaminated retention tank represents the second barrier. The tank downstream of the autoclave serves merely for containment in emergencies. In the event of any contamination, it should be disinfected. Continuous methods are only advisable if the effluent is produced in large quantities and also continuously. They require costly process control and should be validated under all circumstances. The construction of such a plant also calls for observance of the above-mentioned twin-barrier principle via a downstream holding tank. Discharge from the holding tank to the drain system may only take place if the inactivation process proceeds in a fault-free manner.

## 9. Selected literature

*The following German industry standards (DIN) and European standards (EN) effective at the time are to be observed in the case of steam autoclaves:*

- DIN 58946 Part 5 Steam sterilizers; small sterilizers; requirements
- EN 285 Sterilization – Steam sterilizers – Large sterilizers

*For technical details of equipment, the following leaflets are work recommending:*

- International Association for Social Security IVSS (1999) Safe handling of biological agents, work in production, Part 3  
Online reference source: <http://www.suva.ch>
- List of disinfectants and disinfection methods tested and recognised by the Robert Koch Institute  
Status as at 15/6/97 (13th issue)  
Online reference address: <http://www.rki.de>
- Leaflet B 002 (1992): Equipment and organisational measures: laboratories, Professional Association for the Chemicals Industry  
Online reference source: <http://www.bgchemie.de>
- Leaflet B 003 (1992): Equipment and organisational measures: operation Professional Association for the Chemicals Industry  
Online reference source: <http://www.bgchemie.de>

*Reference books with extensive lists*

- Steuer, W.; Lutz-Dettinger, U.; Schubert, F. (1998) Leitfaden der Desinfektion, Sterilisation und Entwesung, Gustav Fischer Verlag, Stuttgart
- Wallhäuser, K. H. (1995) Praxis der Sterilisation, Desinfektion, Konservierung, Georg Thieme Verlag, Stuttgart
- Federal Office of Public Health: Biosafety manual for diagnosis – laboratories (in preparation)