Principles of LTSF Sterilisation

Practical tips from the Sterilisation Section of the German Society for Hospital Hygiene – DGKH e.V.

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1 Description of the Low-Temperature Steam and Formaldehyde (LTSF) Process

The LTSF process is a modified steam sterilisation process that uses steam with the addition of formaldehyde as a sterilant (active agent). Accordingly, this is not a form of gas sterilisation but of "condensation sterilisation" based on steam that contains formaldehyde (LTSF process) and which is carried out only with steam at sub-atmospheric pressure.

2 The Different Steps of the LTSF Process

Each LTSF process consists essentially of three phases:

2.1 Conditioning phase

2.2 Sterilisation phase

2.3 Desorption phase

These three phases comprise seven steps, or eight if re-aeration is included, designated as steps (a) to (h).

2.1 Conditioning phase

a) Air removal

b) Steam penetration

c) Condensation of saturated steam at the site of action

d) Formaldehyde enrichment in the condensate film

2.2 Sterilisation phase

e) Sterilisation with different temperatures and corresponding steam pressure values, formaldehyde concentrations and hold times

2.3 Desorption phase

f) Desorption of formaldehyde

g) Drying

h) Re-aeration

3 Description of the Conditioning Phase

Three steps (a) to (c) of Section 2.1

3.1 Vacuum test (leakage test)

A vacuum leakage test must be an integral part of each process; provision must be made for carrying out this separately for maintenance purposes. Leakage tests before conditioning or for maintenance purposes are conducted at the lowest, process-mediated, absolute pressure (as per the manufacturer's instructions) with a test time of 1 min (≥ 0.5 mbar/min) following a preceding pressure stabilisation time of < 15 min. The total test time is around 30 min.

Provision must be made for an ongoing leakage test throughout the entire process cycle to detect any leaks and/or impermissible increase in the steam pressure.

3.2 General

All LTSF processes carry out conditioning by means of a pulsed vacuum that provides for air removal, steam penetration, conditioning of saturated steam on all surfaces to be sterilised, and thus for heating the sterile supplies to the temperature specified by the manufacturer.

Only slight differences in pressure are manifested here (differential-pressure process).

The lower process-mediated value is a function of the temperature of the water used to operate the vacuum pump. The upper value is determined by the steam pressure of the water at the time the operating temperature (50–78 °C) is reached and is in the range 120 to 450 mbar.

In order to be able to completely remove the air and provide for adequate steam penetration (and to an extent for formaldehyde transport), between 10 and 20 vacuum cycles are needed.

To furnish proof of air removal and steam penetration, 3 hollow-A PCDs (as per DIN 867-5) (helix model), each wrapped in two layers of transparent sterilisation packaging as per DIN EN 868-5, are run as a partial load together with suitable biological or chemical indicators.

3.3 Air removal (step a) of 2.1)

Complete air removal is indispensable for adequate steam penetration and for uniform condensation of the saturated steam on all surfaces to be sterilised (see also 3.2).

Hollow devices of different length and thickness react differently to air removal in the LTSF processes than in the steam sterilisation process, e. g. at 134 °C.

Steam sterilisation process at 134 °C:

The longer the tubes and the greater their diameter, the poorer the air removal and the steam penetration (the product of length and diameter serves as the evaluation criterion).

LTSF process at 50 or 60 °C:

The greater the diameter of a tube, the better the air removal and steam penetration. Air removal is very difficult for a diameter ≤ 1 mm (the quotient from length and diameter serves as the evaluation criterion).

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The penetration resistance of the packaging to air in a dry and moist state must be borne in mind.

3.4 Steam penetration (step b) of 2.1)

Steam penetration is a function of complete air removal and is the chief determinant of uniform condensation of the saturated steam or of the steam undergoing saturation from the active solution on all surfaces to be sterilised (see also 3.2 and 3.3).

The penetration resistance of the packaging to formaldehyde-containing steam must be borne in mind.

3.5 Condensation of saturated steam on all surfaces to be sterilised (step c) of 2.1; see also Section 5

Complete air removal and steam penetration are preconditions for uniform condensation of saturated steam on all surfaces to be sterilised (site of action). The aim is to create a "moist film" on all these surfaces. The condensate present on all surfaces is, in turn, indispensable for formaldehyde saturation to be generated within it. The hydrophilic and hygroscopic properties of the packaging must be taken into account in respect of formaldehyde saturation. Hydrophilic packaging acts as a filter for the formaldehyde or as an additional barrier. Examples of hydrophilic packaging include paper and nonpolar synthetic packaging.

4 Classification of Different LTSF Processes Based on Formaldehyde Enrichment in the Condensate Film

Apart from the type of air removal, steam penetration and condensation, the type, time and method of transport (e. g. through the packaging) of formaldehyde are decisive for the distribution (e. g. convection) of the sterilant on all accessible inner and outer surfaces, and hence also for the divergent levels of efficacy manifested by different LTSF processes. Formaldehyde enrichment in the condensate film varies greatly in accordance with the process control mechanisms employed by the different manufacturers (for example, the German market is being currently supplied by 4 manufacturers). The only difference between all the processes listed is in the type of formaldehyde saturation of the condensate on the surfaces to be sterilised. It is recommended that a PCD (e. g. receptacle for tubes), corresponding to the sterile supplies and containing suitable biological or chemical indicators, be used in order to be able to evaluate the respective penetration properties of the process.

4.1 Conditioning with steam and a single injection of formaldehyde at the end of the conditioning phase

During the conditioning phase the medical devices are rendered warm and moist by means of pulsed vacuum procedures while supplying steam. Then a high percentage formaldehyde solution (e. g. 20–40 %) is injected into the steam during the last pressure come-up time at sub-atmospheric pressure.

Since the pressure and temperature profile specified by the manufacturer is reached at the end of the conditioning phase, the differential pressure needed for optimal distribution of the formaldehyde is lacking. Only conditional convection of the formaldehyde (similar to the flow pattern in steam) takes place and hence no reliable penetration to all inner and outer surfaces.

Because the concentration of formaldehyde is less by around a factor of 10,000 during the gas phase than in the liquid phase, the gas-phase process must saturate with formaldehyde all surfaces that have been wetted with condensate. This procedure is very time consuming and depends largely on the process control, because in the gas phase the process of formaldehyde enrichment until the condensate film is saturated with formaldehyde on the surfaces to be sterilised unfolds very slowly.

Efficacy: no problems are manifest in the case of solid instruments if enough formaldehyde is introduced into the chamber under controlled conditions. In tubes and hollow devices formaldehyde is intercepted by the moist inner surfaces at the ends of the tube when it enters the lumen, hence it takes a very long time until the formaldehyde reaches the inner areas of the hollow instruments and tubes. Therefore the process has limitations and difficulties when used for hollow devices. The hollow device model based on DIN EN 675-5 (helix model) is a PCD that is particularly difficulty and, as borne out in practice, can be served only by optimal sterilisation processes.

4.2 Conditioning with steam followed by several formaldehyde injections

To enhance formaldehyde penetration of processes as per 4.1, instead of a single injection shortly before the end of the conditioning phase, concentrated formaldehyde is injected into the steam during several pulsed vacuum procedures (e. g. during the last 4 vacuums).

Efficacy: repeated administration of formaldehyde injections enhances the process described in 4.1. This also holds true for the penetration of formaldehyde into tubes and hollow devices.

4.3 Conditioning with formaldehyde-saturated steam from one vessel or different vessels (vessel evaporation)

To further enhance formaldehyde penetration into hollow devices, instead of water an approx. 2–3 % formaldehyde solution is used for steam generation. This has the advantage that in addition to air removal and steam penetration, formaldehyde penetration takes places simultaneously during the 10 to 20 air-removal cycles. However, it has the disadvantage that the formaldehyde concentration declines during the pulsed vacuum phase in the evaporation vessel and that at the end of pulsed vacuum procedure the formaldehyde concentration will have declined in the active solution which has yet to evaporate. To curtail this effect, provision can be made for evaporation of the active solution from several vessels.

Efficacy: sterilisation of long, narrow volumes has been improvement vis-à-vis 4.1 and 4.1. The decline in the concentration of the active solution is also adversely affected by the chamber load.

4.4 Conditioning with formaldehyde-containing saturated steam by injection (injection evaporation)

In this process, too, the formaldehyde solution is used already during the pulsed air removal. But to avoid a change in the formaldehyde solution during the condi-

tioning phase, the formaldehyde-containing active solution is supplied directly to the process via injection evaporation. Hence the composition of the evaporated active solution remains constant throughout the entire process. This provides for an ongoing uniform formaldehyde concentration in the gas phase within the chamber which is not dependent on the load.

Efficacy: sterilisation of long, narrow volumes is improved vis-à-vis 4.1 and 4.2.

4.5 Conditioning by addition of steam- and formaldehyde-saturated air

In this process air is conveyed through a concentrated formaldehyde solution, with the air thus becoming enriched with steam and formaldehyde. A pulsed vacuum procedure is conducted with this mixture.

Efficacy: it has been demonstrated that due to the small steam and formaldehyde quantities, these condense on readily accessible surfaces and that dry formaldehyde-free air pockets are formed inside narrow lumens; hence satisfactory sterilisation cannot be carried out at such sites. This method is thus suitable only for solid instruments and has in the meantime been withdrawn by manufacturer because of the inadequate efficacy in hollow device systems.

5 Description of Formaldehyde Enrichment in the Condensate Film (step d) of 2.1

Formaldehyde enrichment takes place in the condensate film on surfaces. This is a vigorous, aggressive form up consumption.

Other terms used here include "formaldehyde saturation in the condensate film"; "reaching the formaldehyde concentration in the condensate film"; "reaching equilibrium between the steam/formaldehyde phase and the formaldehyde concentration in the condensate formed on the surfaces" or "diffusion of formaldehyde in the condensate film".

Enrichment is based on chemical bonding of formaldehyde and water (Fig. 1).

Formaldehyde binds in a similar manner to cellulose or the cell-wall components of microorganisms.

Formaldehyde saturation in the condensate film is a function of the temperature and process (how and when formaldehyde is added) and can show enormous temporal variance.

Saturation is determined in any individual case by the partial pressure values (e. g. steam and formaldehyde phase).

An ideal equilibrium, i. e. equilibrium between ad- and desorption processes, is rarely achieved in practice; rather a "defined unequilibrium" prevails.

Based on Walker and Lacy (1994), at "equilibrium" at around 60 °C a saturation concentration of approx. 1 mol/l (corresponds to around 2 percentages by weight of formaldehyde) in the condensate film at the site of action is derived from a formaldehyde content of approx. 2 mmol/l (corresponds to around 3 percentages by weight of formaldehyde) in the steam gas phase.

In accordance with Gömann et al. (2000), the formaldehyde concentrations at equilibrium between the liquid and gas phase differ by about the factor 10,000 (the difference fluctuates depending on the temperature between 7,000 and 10,000), i.e. 2 percentages by weight of formaldehyde in the condensate film on the surfaces mean 20,000 times fewer percentages by weight of formaldehyde in the gas phase. This thus expresses as a quantity the phenomenon underlying enrichment. If possible, the formaldehyde concentration should be measured in the condensate and not in the gas phase.

Hitherto, only the relationship between 2 substances (saturated steam and formaldehyde) has been examined. But in general a three-substance mixture is involved here because alcohol (alcohol/methanol e. g. 3%) is added to stabilize the formaldehyde. Alcohol has a high partial pressure and the alcohol content increases the pressure by around 10–20 mbar, whereas the formaldehyde increases it by only around 3 mbar. At 60 °C, a pressure increase of 8 mbar corresponds somewhat to a temperature increase of 1 °C. This means that the partial pressure values of alcohol account for around 1–2 °C and those of formaldehyde for around 0.5 °C. When carrying out parametric measurements this could incorrectly simulate the presence of a non-existent saturated steam condition, of overheating or of overshooting of the temperature range in the case of pressure-controlled processes when using, for instance, higher alcohol values of around 10 % alcohol. Therefore when evaluating the theoretical saturated steam temperature, the partial pressure values must be taken into consideration.

6 Description of the Sterilisation Phases (step e) of Section 2.2

The sterilisation phases differ from each other in the different processes in that different hold times are used, depending on the temperature and the effective formaldehyde concentration on the device.

Most processes ensure that during the sterilisation time the temperature is maintained within defined limits under steam saturation conditions from the active solution. Furthermore, process control must be managed such that the formaldehyde concentration in the condensate film is sufficient at all sites of action for effective sterilisation. Therefore provision must be made for a moisture film with the active formaldehyde concentration during the hold time. This is done for example by means of constant barometrically compensated pressure-controlled feeding of the steam-formaldehyde mixture in the saturation range throughout the entire hold time, while at the same time removing the collected condensate from the chamber. In this way the formaldehyde consumed by the process is replaced and drying prevented, (e. g. "deactivation" by means of hydrate formation, "consumption" in packaging on

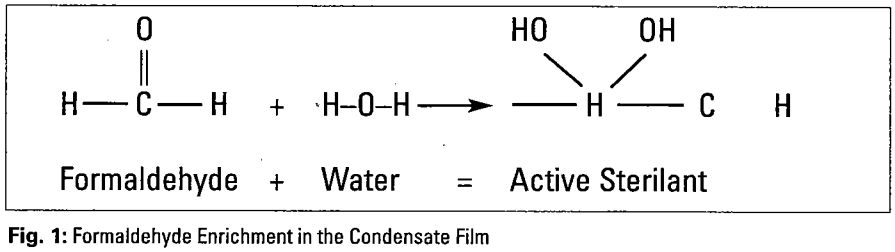

Fig. 1: Formaldehyde Enrichment in the Condensate Film

the journey through the hollow device or by water accumulation).

Example of the sterilisation phase based on a profile specified by the manufacturer for the temperature, pressure and time for a formaldehyde content of 2% in the active solution for the hold time: 60 °C, 200 mbar abs., 60 minutes for an overall load time of 3–4 h, depending on the mass of the load.

The duration of the hold time is the chief determinant of sterility assurance.

The hold time begins just after expiry of the compensation time. This is the period of time during which the parameters of relevance to the process within the chamber must be maintained within specified tolerance ranges. In practice, the terms "hold time" and "exposure time" are used interchangeably.

The compensation time

Compared with steam sterilisation, in the LTSF process the compensation time can be divided into two periods: one period comprises the air removal and steam introduction with formation of the condensate film at all sites of action; the other period comprises the introduction of formaldehyde into all parts of the load and saturation of the moisture film. Depending on the method of formaldehyde introduction used, there can be overlapping of the two compensation times.

For this reason indirect detection of formaldehyde saturation at the site of action might be preferable to concentration measurement in the gas phase. For example, such a test could be conducted with a porous filter paper (e. g. chromatography paper) in a suitable receptacle. A conceivable procedure would be: weigh the dry paper, position four receptacles with a technical mechanism for time-coded safe closure (e. g. 1–2 times before the plateau time, during the plateau time and at the end) at different locations in the chamber. Calculate the weight of the paper (moisture absorption) and hence titrate the formaldehyde.

The given compensation time for saturation expires, or could expire, later than the compensation time for the measured temperatures.

Reaction kinetics:

Based on Spicher and Peters (1981, 1995), the kill kinetics for microorganisms in formaldehyde solutions is a function of the temperature, concentration and microbial count. According to Gömann et al. (2000), a reaction kinetics of the second order takes place.

In view of these insights, the new standard for biological indicators is no longer governed by DIN 866-5 in a gas phase but as specified hitherto by DIN 58948 Part 14 in a formaldehyde suspension.

Process assessment:

To assess the process, the kill curve must be known. Since saturation in the condensate film is mediated by, among other things, how and when formaldehyde is added, different kill curves are also seen.

If steam and formaldehyde are added simultaneously, the kill curve is linear using a semilogarithmic illustration.

If the formaldehyde is added at a later point, there will be a delay in microbial inactivation.

Hence it is not possible to check the latter processes in either the direct half cycle or in partial cycles. They must be checked in the full cycle while using suitable biological indicators (e. g. with different F-biological values). Chemical indicators are not suitable to this effect. Partial cycles can be used for verification purposes only if a linear kill curve can be expected from the process control. If the course of the curve is known from the type test, two points on the kill curve may be enough in certain cases for process assessment. If the course of the curve is not known, more than three points should be available for assessment. This test method may be required for instance for special items or to verify certain aspects of validation.

7 Description of Formaldehyde Desorption (step f) of Section 2.3

Formaldehyde is washed out with steam.

In all processes the sterilant formaldehyde is reliably removed from the sterile items by means of several steam pulsed vacuum steps. The number of such pulsed vacuum procedures will depend on the actual difference in pressure prevailing at the time of steam supply and evacuation. Any significant residues of formaldehyde can be easily detected after opening the steriliser because of a lingering formaldehyde odour. This is because the human nose is able to smell formaldehyde at an even 500-fold lower concentration than ethylene. (Packaging based on cellulose paper is also less conducive to desorption than e. g. synthetic materials such as Tyvek).

8 Description of Drying (step g) of Section 2.3

Drying can be achieved for all processes by engaging in cyclic, pulsed aeration and air removal (renewed vacuum followed by flushing with sterile air). The degree of drying can be reliably estimated by weighing the sterile supplies before and after sterilisation. If moisture has not been adequately removed, this will be olfactorily manifest at the time of withdrawing the sterile supplies.

9 Re-Aeration (step h) of Section 2.3

Re-aeration is an additional service provided by the steriliser manufacturer for some processes to further reduce formaldehyde residues in/on packaging materials. This measure can be interpreted in the light of efforts undertaken to minimise hazardous substances. The steriliser can be safely opened already after desorption/drying (steps f) and g) of Section 2.3).

10 Risks Posed by All LTSF Processes

10.1 Supplies too warm

If the sterile supplies are too warm, uniform condensate formation on all surfaces to be sterilised cannot be guaranteed even if provision has been made for effective air removal and steam penetration. This moisture film is, in turn, a basic precondition for adequate incorporation of formaldehyde. A further problem arises if a lot of condensate with formaldehyde is to be found on the chamber walls and floor. This means that the process is being denied this formaldehyde.

Possible reasons for this:

- too much heat radiation from chamber wall

- preheated supplies
- preheating of supplies because of prolonged storage in the heated chamber before starting the process and/or because of prolonged evacuation times, especially in the initial pulsed vacuum procedures.

It must therefore be ensured that:

- the baseline temperature of the sterile supplies is kept sufficiently low (room temperature),
- supplies are not heated by the chamber wall
- the sterile supplies are heated only by means of condensation to the sterilisation temperature specified by the manufacturer.

For parametric measurement, the temperatures of the steam (free chamber space), sterile supplies and chamber wall are available. Furthermore, in the event of no or of inadequate condensation on the overheated supplies, insufficient heating of the chamber walls or of parts thereof will alert the operator to the fact that no, or only an inadequate amount of, or indeed too much active agent is being supplied.

10.2 Condensate collections in gaps and narrow hollow devices

If condensate collects in gaps or narrow hollow devices, only a relatively small free surface will be available for formaldehyde enrichment. Hence formaldehyde absorption will be delayed and equally saturation (required active concentration) will be delayed or will not take place at all. In the case of steam sterilisation, this water exerts less influence because in general it is at the same temperature as the enclosed item. Conversely, condensate collections in the LTSF process give rise to increased consumption of formaldehyde from the steam, with attendant repercussions on the desorption profile.

Hence the chamber load must be optimised so that condensate collections are avoided. This can be done by positioning the sterile supplies such that reliable provision is made for draining the condensate. Instructions to that effect can be included in the packing list or a photo can be enclosed in the packaging.

10.3 "Formaldehyde consumption" by packaging and by long hollow devices

If the formaldehyde has to embark on a long trajectory and comes into contact with water (condensate film), the formaldehyde content can be reduced. Formaldehyde consumption takes place in accordance with the length of the pathway it has traversed. This could be for example various forms of packaging (e. g. 2- or 3-fold packaging) or long "pipes" with pronounced condensate film. The opened area in each case intercepts the formaldehyde and, despite subsequent feeding, it makes it harder to convey the formaldehyde as far as the end of the lumen.

It is therefore difficult to sterilise narrow and long hollow devices which, as a result of their specific heat, large mass and poor heat conduction, collect a lot of condensate on their walls.

If only pure steam is used for process conditioning, the formaldehyde will be able to penetrate into long hollow devices only after relatively long periods of time, which are rarely or never complied with in terms of the process operation. Therefore procedures that use formaldehyde already during the pulsed vacuum procedures of the conditioning phase should be used preferentially for hollow devices (see also Section 4).

10.4 Overheating

Steam overheating must be avoided because this could prevent steam condensation on the surfaces of the items undergoing sterilisation. This is because heat is being radiated but without any condensation taking place. Overheating can occur only if the chamber walls or the steam are too hot. Overheating because of hygroscopic condensation is omitted here as in this case no textiles are being sterilised. By measuring the pressure and temperature, while bearing in mind the theoretical temperature calculated from the respective steam pressure, proof should be furnished that saturated steam conditions are prevailing at all surfaces that sterilisation is not taking place within the overheating range.

10.5 Puddle formation

Saturated steam and a stable steam pressure are important preconditions for uniform saturation of formaldehyde in the condensate film so as to, in turn, provide as far as possible for an adequate moisture film on all surfaces to be sterilised. In this context the question arises as to how wet the saturated steam can be. Wet steam promotes condensate formation. In general puddles will contain less enriched formaldehyde than that needed for saturation. Sterility is not assured at any such sites.

11 Important Differences of Relevance to the Process between LTSF Process and Pulsed Prevacuum Process in Steam Sterilisation Processes

- The repercussions of any deviations from the specified temperature range are not so adverse at 50–80 °C as in a steam sterilisation process, so long as the theoretical temperature coincides with this change in temperature.

- Since here no textiles are being sterilised, the problems emanating from hydroscopic condensation do not essentially arise for the LTSF process. But small quantities of porous materials (such as foam materials) can be sterilised.

- Inert gases (non-condensable gases) have less impact on LTSF processes. Mini portions are used for subsequent feeding.

- The fact that processes are constantly conducted under sub-atmospheric pressure gives rise to leakage problems in the LTSF process. Therefore ongoing leakage tests are necessary. Likewise, the use of a suitable PCD for batch control is recommended for monitoring purposes.

At a temperature of 70 °C, biological indicators need less moisture to inactivate spores than do chemical indicators for display purposes. This means that when chemical indicators change colour, biological indicators will do so also. Note: no standardised chemical indicators are available for "resetting the F_0 value".

- When evaluating the temperatures measured and the theoretical saturated temperature for LTSF processes, only the partial pressure of the steam is taken into account in practice. But really the increases in temperature mediated by the

partial pressure of the formaldehyde and especially of the alcohol (formaldehyde stabilizer) should be subtracted. With 3 % alcohol, a correction factor of around 1 °C too high a temperature measurement should be taken into consideration. This is somewhat within the error margin.

– Reference points: because the LTSF process is conducted at sub-atmospheric pressure it is not possible to position a temperature sensor in the condensate flow pipe, as the latter is not used here. The steriliser manufacturer must specify the position that is to serve as reference point, e. g. beside the chamber floor beneath the floor of the useable chamber volume.

– For the LTSF process not only the compensation time for the temperatures but also for saturation of the formaldehyde in the condensate film must be borne in mind. The latter will generally be later than the former.

– It is not possible to extrapolate the D values obtained for biological indicators in steam sterilisation to an LTSF process (see Gömann et al. 2000).

– Hydrophilic packaging acts as a extra barrier to formaldehyde penetration so long as saturation on/in the packaging has not occurred (e. g. paper). Hence when examining the same kind of items, always select the items in multiple packaging with hydrophilic components, if these are being used in practice. Paper is always more hydrophilic than non-polar synthetic packaging. Formaldehyde is able to penetrate hydrophobic packaging unimpeded.

– Critical load: a partial load can be particularly critical in steam sterilisation. Conversely, a full load can be more critical for a LTSF process.

– For certain LTSF processes using formaldehyde injection, the injection needle may be blocked. In such a case, the needle must be flushed with e. g. acetic acid.

– A more pressing consideration than in the case of a steam sterilisation process, the penetration kinetics of the formaldehyde must be monitored on all surfaces of the items to be sterilised. This can be done only by using appropriate indicators for the LTSF process which have been specially processed in suitable PCDs. ✦