Moist-heat sterilization of blood bags

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ABSTRACT: This article provides basic information on the sterilization of blood bags systems by moist-heat. Problems of pressure compensation and steam penetration into the system parts without water inside, and the process choice between single and double autoclaving are discussed.

KEYWORDS: moist-heat sterilization, counterpressure sterilization of blood bags, pressure compensation, steam penetration, autoclaving, autoclave, single or double autoclaving

In their complete configuration, *Blood Bags (BBs)* are sets of four bags; there are also single, double and triple sets. The bags are constituted by bonded and plasticized PVC film, heat-sealed along its perimeter. The tubing is made of thicker PVC; the elements for connection, flow control and sampling are made of polypropylene or polycarbonate. The donation needle is made of stainless steel.

Two bags of the same size contain the *anticoagulant* and the *nutrient* solutions. The other bags, generally smaller (satellite bags), are initially empty and dry; they are isolated from the main bags by closed break-valves. The amount of air in the bags is left as small as possible. A label is pasted onto each bag and bears manufacturer information. The set of bags is protected by an outer package (bag or blister).

The complexity of BBs and the critical nature of their use inevitably lead to the choice of moist-heat as sterilization method. Due to a combination of heat- and pressure-related effects, a traditional sterilization process with initial vacuum and pure saturated steam would cause deformation of the PVC bags and alteration of the solutions contained in them.

The most suitable type of autoclaves for sterilizing blood bag systems are autoclaves in which sterilizing medium is a mixture of steam and air. This method, one of the counterpressure types of sterilization, is also called 'air-over-steam'. It allows controlling the sterilization pressure independently of the temperature: this independence is impossible with conventional pure-steam autoclaves, as the temperature and pressure of pure saturated steam are linked by a one-to-one relation.

An appropriate choice of the sterilization counterpressure is a fundamental factor in avoiding deformation and breakage of the bags and allows compensating for the overpressure inside the bags that contain solutions and air. With the air-over-steam method, it is possible to sterilize at 121 °C with a pressure of 3.0 abs bar in the chamber instead of the pressure of only 2.05 abs bar that corresponds to pure steam at 121 °C.

The mixture of steam and air tends to separate out, as air is approximately 1.6 times denser than steam in equal T and P conditions, but fans on the chamber ceiling contrast this effect. Fedegari fans are magnetically driven, to avoid any risk of leakage along shafts.

To sterilize BBs, autoclaves are recommended which are capable of also performing pure saturated steam programs, with full vacuum and a steam-heated jacket. The slight extra cost makes autoclaves more flexible both for the specific treatment of BBs and for any other materials or products.

In normal conditions, the bioburden of a BB set is expected to be routinely lower than 10^2 cfu and free of microorganisms with D-value higher than 1'. To develop the process, let us assume cautiously that the bioburden is as high as 10^4 cfu.

For obtaining a PNSU (or SAL) of 10^{-7}, ten times better than the value of 10^{-6} prescribed by various Pharmacopoeias and Standards, the coldest point of the load should receive a routinely heat dose corresponding to a F_0–value of no less than 11'. It is important to note that *sterility inside empty bags cannot be demonstrated simply on a thermometric basis.*

In fact, the effectiveness of moist-heat sterilization is based on the presence of moisture in contact with the microorganisms to be inactivated. It is not sufficient to reach and hold the sterilization temperature: water must also be present, as steam or as liquid. To obtain evidence that moist heat is obtained also inside the empty bags and in the tubing, a microbiological technique must be used and suitable *bio-indicators of the dry type* be placed inside the empty spaces before they are closed.

There are two approaches to achieve a F_0–value of 11'. To avoid heat damage to the PVC, the first one uses sterilization at the relatively low temperature of 114-115 °C, at which the lethality factor is only 0.20: this entails a rather long exposure time, about 50 minutes. However, there are now PVC types, which withstand a temperature of 121 °C. Moreover, modern air-over-steam autoclaves avoid pressure stresses affecting the BBs, and their sophisticated temperature control eliminates any overheating of the sterilizing medium.

Organic solutions undergo thermal degradation, but the thermal degradation rate varies with the temperature approximately 4 times less than the moist-heat sterilization rate, so it is possible to increase the sterilization temperature, allowing a drastically reduction of its duration: the same F_0–value is obtained at a higher temperature with a less thermal degradation than at a lower temperature. The same principle is used in UHT treatment of milk. These facts allow BB sterilization at 121 °C with exposure time of 11': by definition, a F_0–value of 11' is obtained.

As noted above, BBs are labelled, and the sets finally packaged. An adhesive is used to paste the labels to the bags; this provides a good medium for the growth of molds, especially along the edge of the labels. This growth is facilitated by the moisture that is present inside the final container, also because the PVC of the bags is rather permeable to gases and vapors.

Two approaches are possible to cope with this problem. In the first one, the BB sets are sterilized without the final container: the sterilization process is simpler thanks to the absence of an additional barrier. The sets are then closed hermetically in their final containers, but the entire packages must be subsequently subjected to a pasteurization treatment to inactivate the molds. This additional treatment increases the complexity of the manufacturing process and introduces another factor of degradation.

In the second approach, the BBs are packaged in their final containers and the entire packages are then sterilized. This second method simplifies the manufacturing process, but causes significant difficulties for the penetration of heat and steam inside the sets. In this approach, sterilization at higher temperature and for shorter time is generally used.