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### ► To cite this version:

C. Thorel, C. Lemarié, J.-P. Lemoine, A.-V. Lebellet-Dehaut, C. Herran, et al.. Study of the sterility maintenance during the storage of cotton cloth wrapped medical instruments after a sterilization cycle performed with a mobile autoclave commonly used in rural areas in developing countries. *Le Pharmacien Clinicien*, 2022, 57 (2), pp.125-129. 10.1016/j.phclin.2021.09.004 . hal-04757659

**HAL Id: hal-04757659**

**<https://nantes-universite.hal.science/hal-04757659v1>**

Submitted on 13 Nov 2024

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**Study of the sterility maintenance during the storage of cotton cloth wrapped medical instruments after a sterilization cycle performed with a mobile autoclave commonly used in rural areas in developing countries**

*Etude du maintien de la stérilité lors du stockage de sets d'instrument contenus dans un emballage en coton après un cycle de stérilisation réalisé dans un autoclave mobile habituellement utilisé dans les zones rurales des pays en développement*

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The authors declare that they have no competing interest.

## **Summary**

The "All American" is a mobile autoclave commonly used in developing countries. The objective of this study was to evaluate the sterility of cotton cloth wrapped instruments up to 7 days of storage. For this purpose, 3 "All American" sterilization cycles were conducted, one cycle weekly during a period of three weeks. Each cycle contained 5 sets composed of three clamps, wrapped in cotton cloth. Sterility maintenance was tested at J1, J2, J3, J4 and J7. The sets were stored in a heating chamber in order to reproduce the storage conditions of tropical climates. Bacteriological and mycological analyses were carried out on the outside and inside of the cotton, as well as on the clamps of each set. Although residual humidity rates up to 12% were recorded on cotton sets after cycles, we did not report bacterial or fungal growth, nor inside the cotton wraps, neither onto the clamps.

These results are encouraging, but should be confirmed by increasing repeatability of sterilization cycle and by varying several parameters influencing dryness and sterility to test reproducibility.

**Keywords:** Humanitarian association ; Limited resources; Mobile autoclave; Sterility maintenance; Sterilization; Tropical climate

## **Résumé**

Le « All American » est un autoclave mobile communément utilisé dans les pays en développement. L'objectif de cette étude était d'évaluer le maintien de la stérilité des instruments emballés dans des champs en coton jusqu'à 7 jours de stockage. Pour cela, 3 cycles de stérilisation dans un « All American » ont été réalisés à raison d'un cycle par semaine pendant trois semaines. Chaque charge comportait 5 champs coton contenant chacun trois pinces. Le maintien de la stérilité était analysé à J1, J2, J3, J4 et J7. Les champs étaient conservés dans une étuve afin de reproduire les conditions de stockage des climats tropicaux. Des analyses bactériologiques et mycologiques ont été réalisées à l'extérieur et à l'intérieur du champ coton, ainsi que sur chacune des pinces. Bien que des taux d'humidité des champs coton soient montés jusqu'à 12%, nous n'avons pas mis en évidence de pousse bactérienne ou fongique à l'intérieur des champs en coton ou sur les instruments médicaux. Les résultats obtenus sont encourageants. Ils devraient être complétés par la réalisation de nouveaux cycles de stérilisation afin d'augmenter la répétabilité, tout en faisant varier les paramètres influençant la siccité et la stérilité afin de tester la reproductibilité.

**Mots clefs :** Associations humanitaires ; Ressources limitées ; Autoclave mobile ; Maintien de la stérilité ; Stérilisation ; Climat tropical

## **Introduction**

Controlling healthcare associated infections (HAI) is a major concern for both patients and healthcare professionals [1]. In developing countries and more particularly in rural areas, material and financial resources limit the possibility of using disinfectants or sterilization equipment. In this context, the “All American”, a mobile autoclave that can be heated by using gas or electricity is commonly used. In its guidelines [2], the humanitarian association “Médecins Sans Frontières” (MSF) recommends a sterilization cycle including a 30 mn sterilization phase at 122°C, and strongly recommends to use the sets immediately after the sterilization with “All American” because of water retention, due to the absence of vacuum pump. Furthermore, the standards consider humidity as a risk of contamination regarding the performance of the wrapping system [3]. However, in rural areas where the patient flow is irregular, the caregivers cannot assume which and how many instruments they would need for a day. Therefore, considering that an “All American” requires nearly two hours for achieving a sterilization cycle, it would be more convenient to store the sterilized instruments during several days. The aim of this work is to assess the sterility maintenance of the sets after storage for up to seven days.

## **Methods**

### *Sterilization procedure*

We conducted three “All American” sterilization cycles, heated by gas, one cycle weekly during a period of three weeks. Each time, five sets of three clamps were sterilized. The instruments were wrapped in 180g/m<sup>2</sup> cotton cloth (30cmx40cm), secured by sterilization indicator tape. Three probes (with Temperature and Pressure sensors) and a Bowie Dick (BD) were placed in the “All American” in order to control the sterilization process. The sets were weighed before and after the cycle, to estimate the level of residual humidity. After the

sterilization cycle, the sets were labelled (Week 1, 2, 3 – Storage Day 1, 2, 3, 4, 7) according to their percentage of residual humidity. In order to be placed in the most unfavourable conditions, the wetter the sets were, the longer they were stored before analysis. Then, after sterilization cycle, they were put into closed cover bags to be brought to the laboratory in less than 30 minutes.

### *Laboratory procedures*

The driest set was processed at his arrival at the laboratory. The set with the highest humidity percentage was processed on day 7. The other sets were processed at day 2, 3, 4 according to their residual humidity (Table I). The sets devoted to be analysed on days 2, 3, 4, 7 were taken out of the cover bags with hands disinfected by hand rubbing with alcoholic solution. Then, they were placed at 30°C, with 70% humidity level in the air in order to recreate most of the climate conditions of tropical developing countries.

To assess the cotton barrier role, the cotton cloth of each set was sampled outside and inside, with contact agar plates (Merck, Darmstadt, Germany) containing Tryptic Soy Agar + lecithin tween and histidine (LTH), by using a specific applicator (bioMérieux) allowing a pressure force of 25g/cm<sup>2</sup> during 10 seconds. The plates were then incubated at 30°C during 72h and culture growth was checked at 24h and 48h.

For each set, two clamps were dipped into 20mL of sterile water in a sterile bottle. Then the bottle was automatically shaken during 30 min. Next, the water samples were inoculated under sterile conditions into an aerobic bacterial blood culture vial (BACT/ALERT® FA Plus, bioMérieux), an anaerobic bacterial blood culture vial (BACT/ALERT® FN Plus, bioMérieux) and a yeast blood culture vial (BD BACTEC™ Mycosis IC/F). Those vials were incubated in the automatons (bacT/ALERT® VirtuO®–BioMérieux) for 14 days. For each sterilization cycle (five sets), negative and positive controls

were carried out. The negative controls were obtained by using a clamp sterilized in the sterilization unit of the hospital, and wrap into normalized packet conform to the NF 11607-1 and 11607-2 [3,4]. The bacteriological positive controls were obtained by using a *S.aureus* (ATCC 29213 reference strain) suspension, which was adjusted to the cell density of a McFarland 0.5 turbidity standard. Two successive 1/100 dilutions were performed to obtain a final suspension ( $10^{-4}$  as compared to the initial suspension). Two swabs were impregnated with this final suspension. A swab was used for basting a clamp, then extraction and cultivation were realised by processing as previously described. The other swab was vigorously stirred into 1mL of sterile water. Then 100 $\mu$ L were inoculated in a blood agar plate for counting microorganisms. For *C.albicans* (ATCC reference strain), two dilutions were performed from a suspension adjusted to the density of a McFarland 0.5 turbidity standard to obtain two final suspensions ( $10^{-2}$  and  $10^{-3}$  as compared to the initial suspension). For each of the final suspensions, two swabs were used: one for basting the clamp and one for determining yeast counts.

## Results

The residual humidity rates of sets are presented on table I. The average residual humidity rate was 4.9% (week 1: 4.9%, week 2: 4.5%, week 3: 5.2%). The sets with the higher humidity percentage (days 7), were all positioned at the top of their charge during processing in the “All American”. The residual humidity of the set positioned at the top was 1.5 to 9 times higher than the residual humidity of the other sets sterilized in the same cycle.

Only the contact plates positioned outside the cotton cloth showed micro-organism growth. The plates placed inside were all sterile. It can be hypothesized that all the micro-organisms isolated were from environmental origin, with the exception of *S. aureus*, which could result from a human contamination during the handling process (Table II).

Concerning clamps, none of the blood culture incubated showed a bacterial nor yeast growth while positive controls showed micro-organism growth with different thresholds for *S. aureus* and *C. albicans*. Blood culture vials were positive for all *S. aureus* positive controls. Bacterial counts on the swabs used to blast the clamp showed between 5 and 10 colonies by plate, corresponding to 50 to 100 bacteria per swab. It can be estimated that the clamps were contaminated by the same number of bacteria. Concerning yeast positive controls, one of the dilutions (D1) allowed to obtain  $5 \cdot 10^2$  to  $10^3$  yeast per swab, whereas the other dilution (D2) allowed obtaining  $5 \cdot 10^3$  to  $10^4$  yeasts per swab. Blasting the clamp with D1 resulted in a yeast growth in blood culture vials only once in three controls, whereas blasting the clamp with D2 allowed the growth in all controls.

## **Discussion**

In our knowledge, this is the first study of sterility maintenance after all American sterilization cycle. This device, although rudimentary, is widely deployed (the exact number has not been found) in hospitals in rural areas of developing countries. This deployment is currently carried out without any scientific data. For this reason, in a first study [5], we performed the analysis of parametric data of an all-American cycle. This analysis validated a 30 minutes sterilization cycle at  $122^\circ\text{C}$ . It guarantees the sterilization of the instruments and legitimizes the place of the “All American”.

In this second study, we recreate “All American” conditions of use in developing countries. The aim was that in the handling and storage of the sets, the hygiene conditions should remain the same as those required in the instructions and procedures for the users of this process.

Wrapping instruments in the “All American” is not recommended because of the absence of vacuum pump that does not prevent residual cotton cloth humidity. For each cycle,

the highest residual humidity rate was recorded for the set placed at the top of the charge, despite covering the charge with a cotton cloth for limiting the phenomenon. Concurrently, the residual humidity is not only a problem in “All Americans” [6] but also with type B sterilizer, and the literature tends to demonstrate an association between humidity and micro-organism growth [7]. Based on these data, users should place the sets to be firstly used at the top of the tank. However, it is noteworthy that according to our results, the residual humidity did not seem to influence the micro-organism growth if the sets are manipulated with clean hands after the cycle, and are then stored in a disinfected area.

Even though the cotton cloth is usually not considered to be a very efficient barrier [8, 9], recording a bacterial growth when sampling outside the cotton but not on the instruments placed inside tend to suggest that the cotton cloth has a usefulness in limiting the contamination of sterilized instruments inside.

The quality of the cotton used for packaging must be taken into account. For this reason, the reference used in our tests is the same as the one used in the hospitals in Madagascar. Moreover, in the case where a small autoclave allows sterilization cycles with porous item (type B or S), the EN 13060 standard [10] specifies that cotton used must be made of pure cotton, with a mass per unit area of approximately 180g/m<sup>2</sup>. Our cotton meets this requirement. The cotton must be bleached, which is not our case, but it is untreated as required by the EN 13060 standard.

We can noticed that other studies did not report any bacterial growth onto instruments wrapped in different materials including cotton [11].

Our study has certain limits. First, several parameters influencing dryness and sterility, have not been all considered in our experiments. Among these parameters, most influential are the thickness of the cotton cloth, the number of sets and instruments in the “All



American”. Another limit is the detection threshold of our experiments, especially concerning yeasts. Indeed, we cannot absolutely be sure that no microorganism was present on the clamps. However, the absence of bacterial or fungal growth inside the sets is consistent with an absence of contamination of the instruments [12].

## **Conclusion**

The results obtained in our study suggest the maintenance of sterility after 7 days of storage of wrapped instrument sterilized in “All American sterilizer”. However, this sterility maintenance depends on many factors, potentially linked to the local environment, the different existing procedures, or the staff training. Although our results are encouraging, additional data about repeatability and reproducibility of the results in other conditions are needed to help each staff working in rural areas of developing countries and using “All American” sterilizer to elaborate the better protocols according to their resources. A precaution would be to resterilize unused sets after 7 days of storage.

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<i>Week x / Day y</i>	<b>Day 1</b>	<b>Day 2</b>	<b>Day 3</b>	<b>Day 4</b>	<b>Day 7</b>	<b>Average</b>
<b>Week 1</b>	1.6%	3.6%	3.9%	6.0%	9.6%	4.9%
<b>Week 2</b>	1.4%	2.7%	2.8%	2.9%	12.5%	4.5%
<b>Week 3</b>	2.7%	2.9%	3.7%	5.1%	11.8%	5.2%

**Table I** - Humidity rate of each set after a cycle in the “All American” sterilizer

Week x –	Cotton cloth (outside)	Cotton cloth (inside)
Day y	UFC / 25 cm <sup>2</sup> (bacterial species)	UFC / 25 cm <sup>2</sup>
W1 - D1	0	0
W2 - D1	20 (CoSN, <i>Bacillus</i> spp.)	0
W3 - D1	0	0
W1 - D2	16 ( <i>S. aureus</i> , <i>Bacillus</i> spp.)	0
W2 - D2	1 (CoNS)	0
W3 - D2	10 (CoSN, <i>Moraxella</i> spp, <i>Bacillus</i> spp.)	0
W1 - D3	8 (CoSN, fungi, <i>S. aureus</i> )	0
W2 - D3	2 (CoSN)	0
W3 - D3	2 ( <i>Bacillus</i> spp., <i>Moraxella</i> spp)	0
W1 - D4	2 (CoSN)	0
W2 - D4	0	0
W3 - D4	7 (CoSN, <i>Moraxella</i> spp)	0
W1 - D7	1 (CoSN)	0
W2 - D7	1 ( <i>Bacillus</i> spp.)	0
W3 - D7	5 (CoSN)	0
Coagulase-negative staphylococci (CoNS)		

**Table II** - Results of colony enumeration on Count-tact<sup>®</sup> plates used for sampling outside and inside the cotton cloths per days of storage