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Experimental comparison tests of different systems for the validation of some parameters of standard and non-standard steam sterilization processes.

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SUMMARY

The Authors have devised a set of steam sterilization tests to investigate to what point the presence of air can alter not only the process, but also the correct control of an industrial sterilization cycle. In addition to the usual autoclave equipment, a control apparatus with special software has been employed. Then, by comparing the results obtained with biological and chemical indicators, F_0 detectors with a standard software and with adapted software respectively, it has been possible to estimate how inaccurate some of the present validation methods are.
With the data obtained from such test, some trend curves showing the real behaviour of the sterilization parameters have first been plotted, then the mathematical algorithms have been elaborated, allowing us to suggest a new approach for the validation and the management of a sterilization cycle.

RIASSUNTO

Gli Autori hanno realizzato una serie di prove di sterilizzazione a vapore al fine di indagare quanto la presenza di aria, durante il processo, possa falsare non solo il processo, ma anche il corretto controllo di un ciclo industriale di sterilizzazione.
Per le prove, in aggiunta a quanto già di corredo all'autoclave, è stata impiegata un'apparecchiatura di controllo, dotata di particolare software; confrontando, poi, i risultati ottenuti con l'ausilio di indicatori biologici, indicatori chimici, rilevatori di F0 con software standard e con software modificato, si è potuto valutare quanto inesatti possono essere alcuni degli attuali metodi di convalida.
Dai dati ricavati dalle prove, sono state impostate per prima cosa alcune curve di tendenza dei reali andamenti dei parametri di sterilizzazione, da queste poi sono stati elaborati degli algoritmi matematici che hanno permesso di ipotizzare un nuovo approccio di convalida e di gestione di un ciclo di sterilizzazione.

KEYWORDS: **Validation, Degermation, Sterilization**

INTRODUCTION

The effects of the presence of air in the sterilization chamber are being extensively investigated, as it is well-known that the presence of an air-vapour mixture leng thens the sterilization times [1-3].
Overheated steam conditions can also change the sterilization times [4].
Modern autoclaves are equipped with various devices to counterbalance anomalous conditions in the process, although it is well known that in practice no sterilization process can be carried on in saturated-steam conditions (i.e. ideal conditions). Indeed, in the majority of cases the steam is either unsaturated or overheated.
Figure 1, representing the vapour pressure at different temperatures shows clearly that working conditions are usually far from the ideal situation, which can only be found along the equilibrium line [5].

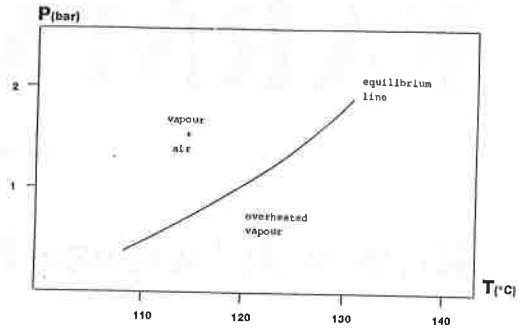

Fig. 1 - Representation of the real vapour condition in a steam sterilization process

Several papers and official publications exist dealing with the parameters to be used (z, D, F_0) to set up and manage a sterilization cycle under saturated-vapour conditions [6-10].
On the other hand, very little can be found in the literature when the conditions are removed from the ideal ones and although the feeling that these parameters can change is widespread, no account is usually taken of this in a validation study or in the management of a process [11].
In this work we aimed at deriving from the actual biology of the sterilization process a set of algorithms for a dynamic evaluation of parameters Tref, D, z so as to relate it to the actual conditions of the process to be used in a validation or management software of a sterilization process.
We began the study of these parameters starting from their values in the two extreme working conditions found in the literature, i.e.
Linear equations have been devised for the evaluation of

parameters	saturated steam	dry heat
T_{ref}	121	170
z	10	25
D	1	5

the intermediate situation starting from these data. From the experimental data derived by means of the biological and chemical indicator [12-14] and the uncorrect to ones we

Table I - Experimental Results obtained from the four sterilization Tests

Test	Thermalog-S band (mm)	Killed Attest spores	F_0 theorical	F_0 from Thermalog-S	F_0 from Attest spores	unsaturation (%)	Temp. (° C)
1	13.4	< E+6	7.336	4.4	< 12	2-4	119-121
2	58.2	> E+6	56.27	19.4	> 12	15	125-126
3	32.8	E+5	39.74	10.9	± 10	20-25	128-130
4	56.0	> E+6	116.4	18.7	> 12	30	130-131

have formulated exponential curves which best fit the experimental conditions and by means of these algorithms have been elaborated and put into the software of our apparatus.
The test and the simulations have been carried out in the Pharmaceutical Technology Laboratory of Siena University.

EXPERIMENTAL

Materials

- 430 liter autoclave parallelepiped form, horizontal type DLOV/22 (De Lama S.p.A.) equipped with SENASYSTEM apparatus for the control of F_0 and with air-steam mixture mixing device.
- Thermalog-S® indicators (PYMAH Corp. Sommerville NY U.S.A.; Exclusive agent for Italy: De Lama S.p.A.), in quality of chemical indicators.
- Attest® 1262P indicators (3M Medical-surgical Division St. Paul MN U.S.A.; agent for Italy 3M Italia S.p.A.), in quality of biological indicators.
- FULL SENA SYSTEM VALIDATION (VER 1.01) software for data observation and processing [5].

Methods

Four sterilization cycle have been carried out. The first has been performed under saturated-steam conditions, by means of pulsing sterilizer cycle system. The other cycle have been performed under programmed increasing unsaturation conditions with cycles which do not propose to eliminate the air present in the autoclave, but can even inject it up to the required pressure.
At each sterilization cycle the following instruments have been placed in the autoclave on a metallic support: n° 1 thermocouple type T, accuracy class 0.05 typical (max. 0.1) connected to the SENASYSTEM apparatus; n° 1 pressure transducer SPI Type 512 2 152, fabr. n° 81/026629 (ECKARDT AG - D) connected to the SENASYSTEM apparatus; n° 5 Thermalog-S® indicators; n° 5 Attest® containers. A software standard having constant values for z=10, D=1 and T_{ref}=121, i.e. not influenced by steam insaturation has been inserted in the F_0 evalutation system.
Attest® spores have been incubated after the treatment suggested by the manufacturer. In the Thermalog-S® the length of the band until the end point has been measured and it has been calculated as the average over the five samples expressed in mm (3 mm being equivalente to 1 F_0; confidence limit = ± 4 mm).
The band length is thoroughly uniform in the five samples.

RESULTS AND DISCUSSION

To make reading easier the data of the four sterilization processes have been summarized in Table I.
The value of F_0 with fixed and constant (Trif, D, z) parameters is given by the device inserted in the autoclave in real time. From the known values of temperature and pressure obtained during each sterilization process, the decrease of the microbial charge has been calculated by

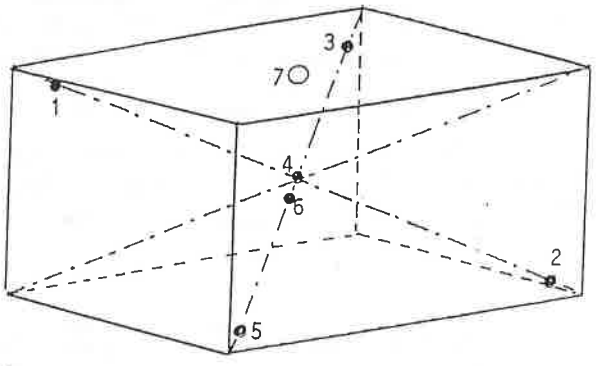

Fig. 2 - Positions of indicators and sensors inside the autoclave: 1-5 Positions of Thermalog-S® and Attest® indicators. 6 Thermocouple. 7 Pressure from lancer.

means of the formula [5]):

$$\Sigma F_O = \Delta_t \times 10^{\frac{T_{rif} - T_{121}}{z}} \times \frac{1}{D}$$

function of temperature alone with no regard to the steam unsaturation parameter. The problem is well known, but the approach to correction is difficult and this has been the main object of our study.

Table II- Experimental Results obtained from the four sterilization Tests

	T_{rif}	D	z
Eqs. 1	$y = 121 + 0.490\,x$	$y = 1 + 0.04\,x$	$y = 10 + 0.15\,x$
Eqs. 2	$y = 121 + \frac{x}{10}^{1.6902}$	$y = 1 + \frac{x}{46}^{1.7853}$	$y = 10 + \frac{x}{21}^{1.7352}$
Eqs. 3	$y = 121 + \frac{x}{20}^{2.4182}$	$y = 1 + \frac{x}{60}^{2.7145}$	$y = 10 + \frac{x}{35}^{2.5795}$

In our elaboration we have allowed the interpolation model of parameters T_{ref}, D, z to vary, as Fig. 2 and Table II show, i.e.:
1) by a straight line;
2) by an exponential of the type $y = x^n$ which has been given two values form, as shown in Table II.
The degree of unsaturation in each measurement in the autoclave, x, is expressed as a percentage and calculated by means of an algorithm consistent with Dalton's law. The values obtained are shown in Table III.

From Table III the following remarks can be made:

i) the way the F_0 regulator is placed on the apparatus prevents it from giving out results bearing a direct correlation to the actual microbial contamination in the process. This depends on the fact that F_0 is evaluated as a

ii) Attest spores, which have a spore charge between $2x10^5$ and $1x10^6$ and a D value between 1.5 and 2.8, as indicated by the manufacturer, can tell whether a process is beyond 12 F_0 (tests 2 and 4) or below such value (test 1). In the test 3 a value of 10 F_0 can be assumed, owing to the colour change of 4 indicators out of 5. The Attest device is sensitive to unsaturated steam.

iii) The Thermalog-S® which are sensitive to unsaturated steam conditions once again prove to be the most appropriate to test the actual sterilization conditions, as they allow a quantitative evaluation in almost all operation conditions (saturated or unsaturated steam). The results obtained even though by means of chemical indicators are consistent with those obtained with Attest® spores (although these do not allow to determine how much below or above 12 F_0 the measurements is) and can give a good quantitative indication.

Table III - Equations devised and used for the evaluation of parameters Tref, D, z in the calculation of the lowering of the microbial charge during a sterilization cycle.

Test	F_0 fixed values	F_0 most probable	F_0 elaboration Eq. 1	F_0 elaboration Eq. 2	F_0 elaboration Eq. 3
1	7.336	4.4	4.343	4.753	5.112
2	56.267	19.4	1.837	14.132	21.434
3	39.743	10.9	2.168	6.452	10.243
4	116.463	18.7	7.143	15.863	22.852

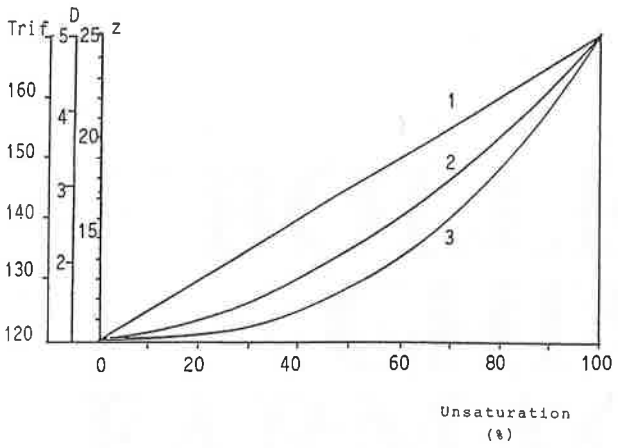

Fig. 3 - Graphic representation of the equations employed to evaluate T_{ref}, D and z.

iv) The result we believe to be worthy of the greatest consideration is the one obtained from the F_0 electronic indicator. Unfortunately, the current systems for the validation and management of a sterilization cycle do not give out values comparable with the actual microbial charge. If the sterilization process occurs under conditions sensibly far from those of saturated steam, values of F_0 are obtained which are totally uncorrelated to the actual process and this fact can make it difficult to decide whether a given cycle is acceptable or not. This is clearly shown in our tests. In particular:

- test 1, being performed under "near-saturation" conditions, approaches an acceptable result.

- test 2-4 being performed under fairly high values of unsaturation, have given an unacceptable set of results.

As we have proposed and carried out, the elaboration of the parameter calculation by means of correction algorithms for T_{ref}, z, D parameters is able to allow a good correlation of the results obtained with the most probable ones, i.e. the ones nearest to the actual microbial charge.

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