A Multiplace Hyperbaric Chamber (MHC) placed in a hospital is subjected to the risks of hospital infection like any other ward in the institution.

If Hyperbaric Oxygen Therapy (HBO) is also done, the chamber may receive more miscellaneous patients than troubled divers. That means: more severe illnesses than persons who were previously healthy before the accident.

Several of those patients may be affected by primary septic diseases (gas gangrene, osteomyelitis, etc.) or other presumably over-infected alterations (open wounds, arteriopathies, etc.).

On the other hand, diving accidents may need relatively large period of hyperbaric treatment inside the chamber and they obviously need life support attentions like alimentation, sleeping facilities and sanitary services.

Finally, the instauration of some medical techniques for critical intensive care in the case of very serious patients inside the chamber, may apport other sources of microbiological contamination (Table I).

<table>
<thead>
<tr>
<th>Septical patients</th>
<th>Medical staff</th>
<th>Medical sanitary equipment</th>
<th>Food remains</th>
<th>Contaminated stock of compressed air</th>
<th>Polluted air procedent from the hospital</th>
</tr>
</thead>
</table>

**TABLE I : Sources of contamination of a hyperbaric chamber.**

In spite of the bacteriostatic effect attributed to the hyperbaric medium (1,2) the possibility of hospital infection in the
MHC is certainly obvious and special techniques of control and treatment are imperative.

The Medical Department of the C.R.I.S. began in 1980 to do HBO in their old MHC, constructed by themselves in 1964, and placed in the Red-Cross Hospital of Barcelona (Fig. 1, pag. 118).

Since that moment, the sporadic utilization of the chamber, in cases of Diving Accidents, was considerably increased accepting patients from several diseases included in groups I and II of the Oxygenation Committee of the Undersea Medical Society (see pag. 150).

Independently of the daily antiseptic procedures, a systematic bacteriological control has been performed, whose results have been reviewed.

**SUBJECTS AND METHODS**

A dual compartment hyperbaric chamber sized 180x380 cms. has been used (Volume = 9.67 m$^3$).

It works every day of the year, during a variable number of hours depending on the number and type of patients (Fig. 2).

Fig. 2: Patient of Gas Gangrene receiving critical intensive care inside the chamber.

A maximum number of six patients occupied the MHC simultaneously (Fig. 3).

The monthly working time depends on the number of patients and the type of diseases treated. This time also varied during the studied period from a minimum of 77 hours/patient/month to a
maximum of 464 hours/patient/month (mean 244.40), accumulating a total present number of approximately 10,000 hours/patient (Table II).

<table>
<thead>
<tr>
<th>Month</th>
<th>1981-82</th>
<th>1982-83</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>194</td>
<td>230</td>
</tr>
<tr>
<td>June</td>
<td>285</td>
<td>219</td>
</tr>
<tr>
<td>July</td>
<td>321</td>
<td>158</td>
</tr>
<tr>
<td>August</td>
<td>175</td>
<td>250</td>
</tr>
<tr>
<td>September</td>
<td>141</td>
<td>100</td>
</tr>
<tr>
<td>October</td>
<td>288</td>
<td>83</td>
</tr>
<tr>
<td>November</td>
<td>273</td>
<td>77</td>
</tr>
<tr>
<td>December</td>
<td>186</td>
<td>308</td>
</tr>
<tr>
<td>January</td>
<td>142</td>
<td>149</td>
</tr>
<tr>
<td>February</td>
<td>156</td>
<td>176</td>
</tr>
<tr>
<td>March</td>
<td>195</td>
<td>360</td>
</tr>
<tr>
<td>April</td>
<td>216</td>
<td>464</td>
</tr>
</tbody>
</table>

**Total**     | **2572** | **2574** |

**TABLE II**: Indexes of monthly occupation of the chamber expressed in number of hours/patient.
The internal temperature of the MHC fluctuated between 19-34°C, and the relative humidity between 40-85%.

Classical cleaning methods have been used throughout routine schedules. Several antiseptics have been used according to the references in the specialized literature (3,4,5).

The surfaces of the chamber itself are cleaned with formaldehyde or sodic hypochlorite solution.

Iron instruments are sterilized by wet or dry heat in autoclave.

The plastic elements, air-oxygen regulators, facial masks, and non heat resistant materials are sterilized with diethylene-oxyde.

Disposable material is preferably used whenever possible. Microbiological samples are taken monthly and also after the presence of septical patients inside the HMC.

The contamination of the air and the surfaces are studied separately.

As standard regulations do not exist in regard to the method of procedure for the study of air (AC) and surface contamination (SC), the method of SEDIMENTATION on Petri's plates has been chosen for the AC, and Roda's contact plates for the demonstration of SC (6).

This decision was made for its advantages of manipulation and performance, for its capability of response to diverse forms of contamination, and its possibility of obtaining quantitative and qualitative values referring to the type of contaminating germs. The Chamber (C) and the Pre-chamber (PC) have been studied separately. In both these zones, a group of three plates of Petri, positioned on the floor in the central part of the zone, were exposed to the environment. Each one of them was filled with a different medium of culture: nutritive Agar, Mac Conkey's medium and Chapmann's medium. The plates were left open during periods of two hours. This period of exposition was considered enough given the reduced air volume to be studied, and taking into account that in hospital zones considered critical and with far greater air volumes, periods of exposition are not usually superior to three hours.

At any rate, the strictness of the method depends especially on the use of periods of exposition which are equal in length, with the aim that all the samples are performed under the same conditions.

The taking of samples was always carried out during the periods of disuse of the MHC, at atmospheric pressure and with the doors closed. Nevertheless, it has been endeavoured that the germs in the sedimentation were the previously introduced by the
patients, staff, air currents, etc. and were not only the vehicular ones.

The taking of samples from the surfaces has been performed by means of Roda's plates filled with nutritive agar. Three plates have been used for each zone (C and PC). Their use is recommended to verify important contaminations and to identify any pathogenic germs, as this type of environmental contamination control, done individually, has no value.

The taking of samples has been performed periodically since May 1981, until the present time with a frequency of monthly intervals. This has been considered suitable given the non-critical interhospital characteristics of the MHC, and always respecting this interval of time in order that the obtained results were methodically comparable. Some revisions not compared have been performed after the use of the chamber of some highly septic patients.

The treatment in the laboratory has been performed under strict regulations of quality bacteriological control. The samples were quickly translated placing them immediately in the incubator (Selecta RT) and maintaining them inside during forty-eight hours at 37°C ± 1.3°C. After this period of incubation, a count of all the developed colonies was performed, obtaining the mean of each group of three plates (PC and C) as quantitative value of AC and the total number per surface in Roda's contac plates.

A microscopic identification by Gram tincture was made for post identification of possible pathogenic germs in the developed colonies in nutritive Agar. In all the developed germs in Chapmann's medium on the plates of Petri that presented positive Manitol, a coagulation test was made for the possible identification of positive coagulasa Stafilococcus. In all the developed germs in Mac Conkey's medium, a sugar test (API) was performed for the identification of Enterobacteriaceaeas and Pseudomonas. Special attention was also given to the presence of Candida (7).

All the material employed in these tests, reagents mediums of cultures, and sterile material, have been submitted to routinary quality controls: sterilization controls of the mediums of culture and sterile material, control of the chemical quality and growth capability of the different mediums of culture, control of the finished medium, sterile manipulation of material, control of the identification reagent, control of the adequate functioning of the incubator, etc.

The statistical valuation of the obtained data throughout these two years of surveillance and bacteriological control, has been made by means of the application of a CHI-SQUARE test
with the original values "number of colonies detected per month in each one of the analysed situations within the MHC" (8).

At the same time, a logarithmical transformation (decimal logarithms) has been made of the values (9) in order to be able to apply the "t" of Student-Fisher and calculate the coefficient of linear correlation of Pearson ($r_{xy}$). The level of statistical significance used is of 5% (6).

RESULTS

We present the qualitative and quantitative results corresponding to the period May 1981 - April 1983.

A. Qualitative Results.

1. A variable number of colonies of non pathogenic non identified germs have been habitually detected.

2. In the control No. 7 (November 1981) two colonies of Pseudomonas Putrefaccies were identified in the air ambit within the PC, which were not found in the samples taken from the surface. Subsequently to this control and immediately after knowing this results, a new control directed to investigate the existance of Pseudomonas was made in both zones, without obtaining positive results. (Probably because after knowing this results intensive cleaning was done).

3. In the control No. 15 (July 1982), a colony of Acinetobacter Calcoaceticus was identified in the air of the C, coinciding with the highest count of non pathogenic germ colonies of all the other ones performed. Immediately, the necessary preventive precautions were started.

B. Quantitative Results.

1. In Table III the intervals of confidence were observed to 99%, of the variable "logarithm of the number of colonies" for each one of the four analysed situations in the studied period.

2. The existence of statistically significant differences among the values of the AC has been confirmed corresponding to the summer season a slightly higher than the samples collected throughout the year, equally in the C as in the PC. These seasonal differences have been in each one of the two studied years (Table IV).

3. The calculation of the coefficient of linear correlation of Pearson between the variables $x =$ decimal logarithm of the number of colonies in the PC, and $y =$ decimal logarithm of the number of colonies in the C, shows the following results : $r_{xy} = 0.6748$. This result is
TABLE III: Confidence Intervals and Means (p<0.01) of the variable "Logarithm of the number of colonies" detected from the air and surface contamination.

<table>
<thead>
<tr>
<th>Zone</th>
<th>Air</th>
<th>Surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prechamber</td>
<td>0.88 - 1.60</td>
<td>0.20 - 1.08</td>
</tr>
<tr>
<td></td>
<td>(1.24)</td>
<td>(0.64)</td>
</tr>
<tr>
<td>Chamber</td>
<td>0.78 - 1.30</td>
<td>0.30 - 1.12</td>
</tr>
<tr>
<td></td>
<td>(1.04)</td>
<td>(0.71)</td>
</tr>
</tbody>
</table>

TABLE IV: Comprobation of the stationality of the distribution of the "number of colonies".

<table>
<thead>
<tr>
<th>Zone</th>
<th>Period</th>
<th>$\chi^2$</th>
<th>p &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prechamber</td>
<td>May 81 - April 82</td>
<td>67.98</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>May 82 - April 83</td>
<td>356.04</td>
<td>0.0001</td>
</tr>
<tr>
<td>Chamber</td>
<td>May 81 - April 82</td>
<td>227.65</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>May 82 - April 83</td>
<td>219.00</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

statistically significant (p<0.01). For this reason, it can be affirmed that during this period of time studied, the number of detected colonies in the air control of the C is related to those detected in the PC. On the other hand, for the analytical determinations of the SC, no statistical dependence exists between the PC and the C during the period of study.

4. The study of the statistical alterations between the AC and SC for each one of both zones separately C and PC, shows no significant relation for the objective period of study.

5. Only in C, significant differences have obtained (p<0.05) between the concerning values of the AC of the first months (May 1982 - April 1983) considered. For this reason, it can be assured that AC in C has diminished during the last months.
DISCUSSION

From the study of the determined intervals of confidence may be observed which are the months that remain outside, and possible explanations may be suggested. Given the importance of the AC, it is necessary to comment that the months which have presented greater contamination (above the superior limit of the interval) equally in PC as in C all coincide with the summer seasons (Fig. 4 and 5). In the same manner, the lowest indexes of contamination (below the inferior limit of the interval) are grouped practically in the last months considered.

![Graph showing air contamination indexes for prechamber]

**Fig. 4:** Indexes of air contamination of the prechamber.

The fact that in the summer season the AC increases (equally in C as in PC) may be motivated by various factors:

- Increase of the number of patients.
- Vehiculization to the environment of a greater quantity of original saprophyte flora of the skin and attached parts, by perspiration and transpiration of the patients and sanitary personnel with few clothes.
- Suitable conditions of temperature and humidity for the proliferation of the germs.
- Door and windows opened to the outside during long period of time.
The existing relation between the AC of C and the AC of the PC indicates that if the contamination increases in the PC, it also increases in the C. Given that the PC is positioned further towards the outside (air streams coming from the hospital, personal passing, etc.) it is necessary to guard it especially in order to control the contamination of the C.

On the contrary to that which was thought on starting these bacteriological control activities, the evaluation of the SC does not contribute to any useful information.

The controls performed on the surfaces of C and PC are not statistically associated (directly and at the same time they are always independent of the air controls. Pathogenic germs have not been found on the surfaces even not when they have been found in the air controls.

**CONCLUSIONS**

The microbiological contamination indexes detected inside the MHC during this two year period, have not been higher than the indexes unfortunately usually obtained in hospitals, and are lower than the indexes obtained from wards of infected patients, or critical care units.

The regular control of the microbiological contamination in the PC and the C is a useful method for the environmental vigilance in the stated zones, specially in the air ambit.

In the last months the indexes of non-pathogen contamination
have been lower than those obtained in equivalent periods of the first year. We attribute this improvement to the amelioration in the aseptic/antiseptic procedures following the advice given for this systematic control.

It is necessary to continue this type of experiences to be able to standardize protocols of microbiological procedures and statistical evaluation.

The exposed procedures and protocols have proved their capability to maintain an acceptable level of infectious innocuity in our chamber.

**SUMMARY**

This is a study of the results of the microbiological contamination control carried out in the Multiplace Hyperbaric Chamber (MHC) of the CRIS, placed in the Red-Cross Hospital of Barcelona, during a period of 24 months. The personal occupation level of the MHC was about 2600 hours/patient. Some septical diseases were treated. The internal temperature fluctuated between 19 to 34°C. The relative humidity between 40 to 85%. The MHC received routine cleaning and disinfection procedures periodically and after every septical patient. **SUBJECTS AND METHODS:** Adequate culture media were prepared monthly in both compartment and samples were taken of air and surface contamination. These were examined for evidence of Enterobacteriaceas, Pseudomonas, Staphylococcus, and Candidas. A quantitative estimation was made of the number of unidentified non-pathogenic germ colonies. Trends and contamination indexes were calculated.

**RESULTS:** There was a demonstrated proliferation of non-pathogenic non identified germs specially during the hot months (p < 0.0001). The air contamination (AC) was greater in the pre-chamber (PC) than in the chamber itself (C). A significant relation between AC of C and PC was detected (r = 0.67, p < 0.01). The study of the surfaces contamination (SC) did not show significant results. On one occasion a Pseudomonas Putrefaciens was isolated from the air of the PC, and another time an Acinetobacter Calcoaceticus was isolated from the air of the C. In both cases the control was repeated and no new growths were obtained. No other pathogenic germs were ever found. **CONCLUSIONS:** A MHC requires effective antiseptic measures to improve the bacteriostatic effect of the hyperbaric medium. The standards presented here have shown to be effective in identifying and maintaining appropriate limits of infectious innocuity.

**RESUM**


S'exposa el resultat de 24 mesos de control de contaminació ambiental microbiològica dut a terme a la cambra hiperbàrica multiplaça (CHM) del CRIS, instalada a l'Hospital de la Creu Roja de Barcelona. El nivell d'ocupació fou de a prop de 2600 hores/malalt. Es van tractar alguns malalts séptics. La temperatura interior va oscil·lar de 19 a 34°C, i l'humitat relativa entre 40 i 85%. La CHM reb mesures de neteja i desinfecció periòdicament i després del pas de cada malalt.

Se expone el resultado de 24 meses de control de contaminación ambiental microbiológica llevado a cabo en la Cámara Hiperbárica Multiplaza (CHM) del CRIS instalada en el Hospital de la Cruz Roja de Barcelona. El nivel de ocupación fue cercano a las 2600 horas/paciente. Se trataron varios pacientes portadores de procesos sépticos. La temperatura interior osciló entre los 19 y los 34°C y la humedad relativa entre 40 y 85%. La CHM recibe las medidas habituales de limpieza y desinfección periódicamente y después del paso de cada enfermo séptico. MATERIAL Y METODO: Se dispusieron mensualmente medios de cultivo adecuados en ambos compartimentos y se tomaron muestras de contaminación aérea (CA) y de superficies (CS). Se investigó la presencia de enterobacteriáceas, pseudomonas, estafilococos, y cándidas. Se estimó cuantitativamente el número de colonias de gérmenes no patógenos. Se realizó un cálculo de tendencias y de índices de contaminación. RESULTADOS: Se evidenció proliferación de gérmenes no patógenos no identificados, especialmente durante los meses calurosos (p<0,0001). La CA fue mayor en la Precámara (PC) y la CS en la Cámara propiamente dicha (C). La CA de la C estuvo en relación con la de la PC (rxy = 0,6748, p<0,01). Los controles de CS no evidenciaron datos significativos de interés. En una ocasión se aisló un germen tipo Pseudonoma Putrefaciens en el aire de la PC y otra vez un Acinetobacter Calcoaceticus en el aire de la C. En ambos casos se repitió el control sin obtener nuevos crecimientos. Nunca más se volvieron a hallar otros gérmenes patógenos. CONCLUSION: Una CHM hospitalaria precisa medidas eficaces de antisépsis para potenciar el efecto bacteriostático atribuído al medio hiperbárico. El protocolo expuesto ha demostrado su eficacia para detectar y mantener unos límites correctos de inocuidad infecciosa.

RESUMEN


On présente le résultat de 24 mois de surveillance de la contamination de l'envi-
ronnement microbiologique réalisé dans le Caisson Hyperbare Multiplace (CHM) du CRIS placé dans l'Hôpital de la Croix Rouge de Barcelone. Le niveau d'occupation a été d'environ 2600 heures/patient. On a traité des malades séptiques. La température intérieure a oscillé entre 19 et 34°C et l'humidité relative entre 40 et 85 %. Le CHM reçoit les mesures habituelles de nettoyage et désinfection périodiqument et après le passage de chaque patient septique. MATERIEL ET METHOD: Des milieux de culture appropriés ont été placés chaque mois dans les deux compartiments, et on a extrait des échantillons de la contamination aérienne et du sol. La présence d'Enterobactéries, de Pseudomones, de Staphylocoques, et de Candides, a été recherché. On a évalué quantitativement le nombre de colonies de germes non pathogènes. Un calcul des tendances et indices de contamination a été effectué. RESULTATS: On met en évidence la prolifération de germes non pathogènes non identifiés, particulièrement pendant les mois de chaleur (p<0,0001). La contamination aérienne (CA) s'est avérée plus importante dans la Prechambre (PC), et la contamination au sol (CS) supérieur au niveau de la chambre (C). La CA de la C et la de la PC ont été relationé (r_xy = 0,6748). Les contrôle de CS n'ont pas donné des résultats significatifs. Un germe type Pseudomone Putrefaciens fut isolé une fois dans l'air de PC, et en une autre occasion un Acinetobacter Calcoaceticus dans l'air de C. Dans les deux cas, le contrôle a été répété sans obtention de nouvelles croissances. Il n'a jamais été trouvé d'autres germes pathogènes. CONCLUSIONS: Un CHM hospitalière demande des mesures rigoureuses d'antisepsie a fin de potentialiser l'effect bactériostatique attribué au milieu hyperbare. Le protocole exposé a démontré son efficacité de détection et mainte-nance des limites correctes d'inocuité infectieuse.

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DIVING AND HYPERBARIC MEDICINE

Proceedings of the IX Congress of the European Undersea Biomedical Society (E.U.B.S.)
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PROCEEDINGS OF THE IX CONGRESS OF THE EUROPEAN UNDERSEA BIOMEDICAL SOCIETY (E.U.B.S.)

Sponsored by
CENTRE DE RECUPERACIÓ I D’INVESTIGACIONS SUBMARINES (C.R.I.S.)

Edited by
JORDI DESOLA ALÀ

EDICIONS C.R.I.S., BARCELONA
IX CONGRESS
of
EUROPEAN UNDERSEA
BIOMEDICAL SOCIETY
(E.U.B.S.)