A History of Isolator and Containment Technology, Part 5: Development and use of sterilising agents with associated devices

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Abstract

In this penultimate section the development of the 'sterilisation' of isolators used for sterility testing and aseptic processing will be reviewed. This will include the chemical agents used, the equipment to deliver and remove that agent (in a gaseous form), as well as agents used for manual wet processes and also fogging techniques with associated equipment.

Sterilising

As mentioned in previous sections of this history the term 'sterilising' has been used when related to the 'sterility' of isolators[1]. A better term perhaps would be 'biological decontamination' but the end result of any such decontamination must be to achieve surfaces and other areas such as filters, apertures and other items placed in the treated environment that should be essentially free of microbial life (i.e. sterile). While some of the agents that will be described have been reported as broad spectrum biocides, their action was initially tested and reported in the liquid phase not as a gas or vapour. The sole exception is formaldehyde which has a long history as a vapour 'sterilising' agent for clean rooms, safety cabinets and animal housing.

Sterilisation methods in the realm of medical or pharmaceutical uses and which have regulatory approval are all described in some detail in the various pharmacopoeias. There are four main methods:

- Moist steam
- Dry heat
- Irradiation (gamma & e-beam)
- Ethylene oxide gas

As none of these have practical use for isolator sterilisation, other agents were sought and these are listed below in an approximate order of their use.

It would be appropriate to mention two other sterilising methods, as agents used for instrument sterilisation have been used in isolators:

1. Low temperature with formaldehyde was used to rapidly sterilise certain instruments, at temperatures ranging from 50°C to 80°C utilising steam. Getinge offered a chamber device based on this type of treatment.
2. Gas plasma sterilisation where the chamber is filled with peracetic acid or hydrogen peroxide vapour under vacuum. After a required exposure time radio frequency energy is applied to the chamber to induce the plasma state in which the active agent is broken down quickly into innocuous parts. This technique is used mainly for instruments and one advantage is that the instrument packs are dry at the end of the cycle. Such a steriliser is the Sterrad® system.

Chemical Agents and Methods

Manual Wet Processes

There are many chemical agents that could be used for a wet process to sterilise an isolator. In the very early days of isolators the following agents were employed:

1. Alcohol-usually 70% concentration. This has the advantage of being easy to use and also kills many vegetative organisms. However has little or no effect on bacterial spores and therefore was not considered as a sterilising agent
2. Aldehyde based products including formaldehyde or glutaraldehyde (Cidex®). Such agents are sporicidal but in many cases require a specific pH range and also a long exposure period. Removal afterwards also created problems.
3. Hypochlorite and related agents. These offer a wide spectrum of activity at a reasonably low cost. Stabilised solutions of chlorine dioxide were also found to be sporicidal but, as with hypochlorites, there were problems with corrosion of metal parts in the isolator.
4. Quaternary ammonium and ampholytic agents. These again were not sporicidal.
5. Phenolic based agents. These were quickly abandoned due to their residual qualities.

The major obstacle to the use of the manual wet processes is that they were not really reproducible on a daily or weekly basis and were difficult to routinely validate and thus convince regulatory authorities of their reproducible efficacy. Their role was relegated to use for cleaning where appropriate, provided that they did not leave residues or react with the sterilising agent.

Fogging or Fumigation processes

Fogging or aerosol devices were developed as alternatives to manual wet processes. Some of these were already in use for treating large volume areas such as clean rooms, operating theatres, agricultural buildings and animal research facilities. Formaldehyde was the main agent used for these purposes – see below.

Recent advances have been the treatment of hospital wards using fogging devices and a solution of hydrogen peroxide with a trace of a silver element. It is claimed that the silver element is part of the lethal effect of the blend.

Products include Sanosil, MICROCHEM M381, EndoSan™, Accepta 8101 (50% H_2O_2) and Accepta 8105 (5% H_2O_2). Some of the higher concentration products are used to treat drinking water but only for a limited

1. Although 'sterility' means free from all living microorganisms, 'sterilisation' is usually stated in terms of probability of survival of a known quantity of a specific microorganism. This explanation is based on definitions given in the ISPE Online Glossary.

period. Some of the suppliers also offer a fogging or aerosol device. These products are not useful for isolator decontamination due to the presence of the silver ion which is not volatile and may present unwanted residues.

Aerosol devices use either a powerful blower or compressed air to create the fog and this presents pressure rise problems when used in an isolator, especially the flexible film type. The general practice was to partially open the exhaust valve to allow the pressure to be stabilised but this led to an escape of the sterilising agent and thus variations in the concentration in the isolator. It was found that a balance on air pressure and the volume of agent fogged into the isolator usually worked without resorting to venting.

An early fogging device produced expressly for isolator use was the La Calhene 'Spram' device. This could be used with any decontamination agent but was largely use with peracetic acid or hydrogen peroxide. The device consisted of a control cabinet and reservoir for the chemical agent. It was connected by pressure tubing to an aerosol head or nozzle fitted to the wall of the isolator. By use of controlled compressed air, an aerosol of the chemical agent was sent into the isolator to the point where it was filled with a 'fog'. Because the air pressure and volume of agent was controlled and measured, it was a reproducible method and could be validated.

Ultrasonic nebulisation

Further advances led to the use of ultrasonic nebulisers and this was seen in a device manufactured by Cambridge Isolation Technology, now Pharminox Isolation. This used ultrasonic nebulisation of peracetic acid to create a fog which evaporated into a vapour before entering into the isolator. The device also had an event recording system for each stage of the sterilising process.

Howorth Air Technology Ltd also uses ultrasonic nebuliser technology in its BioGen™ sporicidal gassing generator, claiming that it is better than hotplate evaporation. The BioGen™ – A (Auto) unit uses 30-35% hydrogen peroxide to produce dry vapour hydrogen peroxide (DVHP™) for "fast and effective balanced high level environmental and surface disinfection of both process enclosures and room volumes up to 1200m^3."

Howorth also manufactures a smaller version, the BioGen™ – M (Mini) unit for microbiological safety cabinets.

Heat plus chemical agents

In the isolator field the only attempt to utilize heat via steam and a chemical agent was described by Pflug et al.[1] [2] It was tested using steam at atmospheric pressure plus hydrogen peroxide. Levels of 1000 to 10,000 ppm of hydrogen peroxide were successfully evaluated against a number of spore forming bacteria. The basic problem appeared to be the requirement to insulate the isolator to prevent undue heat loss.

Gassing or vaporising processes

It was obvious in the late 70s that some form of gassing an isolator was probably the best system to explore. The isolators of that time were mainly flexible film types, which could be sealed, gassed and then aerated using the isolator's own systems. Early in the development of gassing techniques it was found that distribution of the vapour was uneven and small fans placed inside the isolator were necessary to achieve an even concentration throughout the volume of the isolator. This has been overcome to an extent by later gas system designs which have devices used at the point of gas introduction into the enclosure. Gas distribution, however, remains an area open to improvement.

Before progressing to a description of gassing processes, the chemical agents used should be reviewed. They are listed here in approximate order of introduction over the history of isolators.

Formaldehyde

This is usually presented as 37%-40% formaldehyde in water, with methanol as a stabilizer, and is known as formalin. An alternative form is as solid – paraformaldehyde powder or 'prills' (pellets). The chemical is an alkylating agent and has a biocidal action similar to ethylene oxide.

A moderately high air temperature is required, as well as a degree of humidity, to get a useful sterilising effect in the gaseous phase. Either formalin solution or the solid form is heated to evolve the gas. This technique is useful for small enclosures such as safety cabinets, etc. In the early days, with the liquid form, something as simple as an electrical kettle was used but more sophisticated devices followed that could be attached to the cabinet. Water was added to elevate the relative humidity in the enclosure.

For large enclosures, such as animal housings (for example broiler houses before re-stocking), the violent reaction of formalin added to potassium permanganate can be used to generate the formaldehyde vapour. Alternatively, the formaldehyde vapour can be generated in the normal way and released into the building using a suitably large aerosol device. The latter method was preferred as the permanganate/formalin reaction is violent and rapid. Several containers, in line, have to be activated by mixing together the ingredients and the technician has to be athletic to race ahead of the gas evolved and get out of the building!! Similar methods were used to decontaminate clean rooms and operating theatres but were later replaced with aerosol techniques. At least 24 hours were required for the gas to be effective.

Formaldehyde is an excellent biocide, being active against bacteria, fungi, viruses and spore forms of bacteria and fungi. The gas penetrates well but it takes time to do this. It has the disadvantage of requiring an RH of between 60% and 90% and a temperature range of 15 to 32°C. In some early isolators for animal use condensation was a problem and it was not uncommon for a white residue of the polymer paraformaldehyde to develop. Formaldehyde is neutralized by the use of ammonia but this also leads to the deposition of paraformaldehyde.

Concerns were raised with regard to the carcinogenic effect of formaldehyde and it has been replaced as a sterilant for isolators by other chemical agents. It is still used for decontamination of safety cabinets and, to the author's knowledge, at least one large aseptic process isolator.

In summary, the problem with formaldehyde decontamination is the length of time for it to take effect. There is also a possibility of condensation if the surfaces are too cold with a final deposition of paraformaldehyde if the concentration is high. It is however a very good penetrative agent and is still used where live microbial or viral suspensions are handled and processed.

Peracetic acid (PAA)

This chemical agent is manufactured industrially either by auto-oxidation of acetaldehyde:

$O_2 + CH_3CHO \rightarrow CH_3CO_3H$

or by the treatment of acetic acid with hydrogen peroxide. A small amount of catalyst is added (usually an acid):

$H_2O_2 + CH_3CO_2H \leftrightarrow CH_3CO_3H + H_2O$

Peracetic acid was normally offered in liquid form as 35% PAA, 30% acetic acid and 10% hydrogen peroxide. The balance was water and catalyst (<1%). It is very corrosive at 35%, moderately corrosive at lower dilutions and a powerful oxidizing agent.

PAA has a wide range of activity against bacteria, fungi, molds, viruses and bacterial spores. In solution it is very rapid in action at low dilution (<1%) and is considered to be a useful sterilising agent for surgical instruments in an emergency.

During the early use of PAA, a 35% solution was diluted before use and had then to be stored at low temperature to prevent a loss of PAA by reversion to hydrogen peroxide. Later formulations of 'ready to use' solutions had concentrations of 3.5% PAA and 9 to 10% H_2O_2 and could be stored at room temperature.

In the development of germ-free animal isolators, diluted PAA (1%) was used for liquid baths for passing wrapped sterile animal food and bottles of water into the isolator room where the animals were held. Trexler reported bacteriological problems in some animal housings and the concentration of PAA was consequently increased to 2%, then 3% and finally 3.5%, which was a ten-fold dilution of the concentrate thus making the dilution easier[3]. 35% PAA is a very hostile chemical and requires all safety precautions when preparing dilutions.

A major use of PAA was the cleaning and disinfection of pipelines and vats used in the manufacture of beer and wine where it also removes biofilm deposits. It has also found similar use in the pharmaceutical industry, concentrations of 9% to 15% being commonly used for this purpose. Other concentrations of PAA are now offered for various uses by Solvay and other manufacturers.

Peracetic acid was found to be volatile when warm air was blown across the surface of a 3.0 to 3.5% PAA solution and, in this vapour form, an excellent biocide for sterilising isolators.[4]

Schülke France SARL now offers Soproper, manufactured by BIOXAL SA – AIR LIQUIDE Groupe. Soproper is a solution of 3.5% PAA with 10-12% hydrogen peroxide. It is stable at room temperature due to the higher concentration of hydrogen peroxide.

One disadvantage of PAA sterilisation in isolators is the residual odour of acetic acid which takes a long time to remove during the aeration period. This is especially noticeable with the flexible film type of isolator.

Hydrogen peroxide

Hydrogen peroxide is offered in several concentrations ranging for 3% to 50%. Higher concentrations are available but as the amount increases so does the hazard for detonation and fire so normally 35% concentration is the highest concentration usually available commercially in bulk. It is a powerful oxidizing agent and has a wide spectrum of activity against bacteria, fungi, molds, viruses and bacterial spores. The speed of activity depends on the concentration and 3% solutions are used for safe skin and wound disinfection.

Hydrogen peroxide is less corrosive than PAA for materials prone to corrosion but there is an explosive risk if it reaches high concentrations.

Amsco (now Steris) started to develop disinfection systems based on peracetic acid and hydrogen peroxide. As hydrogen peroxide degrades to water and oxygen it was an obvious choice as a potential sterilant for isolators.

Initially Amsco (Steris) developed a small chamber using vaporised hydrogen peroxide for sterilising dental instruments but expanded that concept to a gas generator for sterilising flexible film isolators using 35% hydrogen peroxide under controlled conditions of delivery. Other companies followed such as Bioquell in the UK. All the earlier units were mobile so they could be moved from one isolator to another but a later development was the integration of the gassing unit into the isolator itself: SKAN in Switzerland and Metall+Plastic in Germany as well as Steris and La Calhene (now Getinge La Calhene) followed this route as an alternative to mobile units.

Chlorine dioxide

This agent is a very powerful oxidizing substance. It is a greenish/yellow gas and at concentrations >30% in air it can dissociate explosively into chlorine and oxygen. Fortunately, for isolator purposes, very low concentrations are used for sterilisation. Industrially it has been used to treat drinking water and for sanitizing certain fruits.

Chlorine dioxide is a very powerful oxidizing agent and has a very wide span of activity not only against bacteria, fungi and viruses but also against small animal parasites and oocysts. It has excellent penetrating properties.

There are several pathways to prepare chlorine dioxide. It cannot be stored as a gas but is generated when required. There are solutions of the agent that are stabilized by the use of acids and are offered for high level disinfection.

From a gas generation view the simplest way is to react chlorine gas with sodium chlorite:

$2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2NaCl$

In 1991 Johnson & Johnson (USA) purchased gaseous ClO_2 sterilisation from Scopas Technology Co., who had developed the technology. From this, a gaseous sterilisation system for isolators and rooms was initiated and was offered as the ClorDiSys process. Today it is marketed by CloDiSys Solutions Inc., who claim it is easy to monitor (the gas generator has a built-in monitor) and, being a true gas, is not affected by temperature unlike vaporised hydrogen peroxide. The cycle does however require a high RH (65%).

Ozone

Ozone has been used for the treatment of drinking water in much of Europe but it has not been investigated sufficiently for use in isolators. It is a powerful oxidizing agent that breaks down quickly into oxygen. It is active against a wide range of organisms but has found its niche in the treatment of water.

There are small ozone generators available but these are mainly employed for producing ozone to be added to water for washing down animal carcases prior to butchering.

The author has experimented with ozone in a small ($2m^3$) isolator with some success in deactivating the spores of *Geobacillus stearothermophilus*.

An interesting review of four of the above agents was performed in the Health & Safety Laboratory, Derbyshire, UK.[5]

Gassing devices

As gassing devices come in many shapes and sizes they will be described for each agent. Gassing is defined as the evaporation of the agent prior to entry into an enclosure or a room or as a true gas at source.

Formaldehyde gas generators

For room treatment there are devices that generate formaldehyde vapour followed by ammonia vapour, all controlled automatically. The ammonia is used to neutralize any residual formaldehyde. Such a device is the Nextek 1414RH model (see Figure 1).

In the use of paraformaldehyde the agent was placed on a hot plate and subsequent heating generated formaldehyde vapour. Variations on this theme were used to decontaminate safety cabinets.

As mentioned, using an electric kettle works just as well for safety cabinets. In evaporating formalin, water must be added in equal volume to create the required high humidity. Pre-heating the environment also increases the lethal effect of formaldehyde and prevents condensation.

One facility with a multipurpose isolator (vial filling and freeze drying) commenced sterilising using formaldehyde vapour. The isolator manufacturer, Metall+ Plastic, constructed a built-in formaldehyde evaporator which eventually worked extremely well. The unusual choice of formaldehyde was because the particular application was the handling and filling of live microbial agents for which the user had much previous experience using formaldehyde.

Peracetic acid (PAA) gas generators

Prior to the use of vaporised PAA, a diluted solution had been used in pass through baths (dunk tanks) for delivering sterile water containers and sterile wrapped animal food into rooms with isolators holding germ free or single germ strain animals. Formaldehyde was used as the room fumigant prior to stocking with the animals and early isolators made of metal had been steam sterilised.

With the advent of flexible film isolators for sterility testing and for other aseptic uses, an alternative to formaldehyde was sought.

La Calhene introduced a simple gas generator for evaporating PAA where compressed air was blown over a heated bowl (40 to 45°C) containing a dedicated solution of 3.5% PAA (Soproper). The PAA was then introduced into the isolator through filters over a period of several hours.

A later version of this called MAN is shown in Figure 2.

This method of gassing proved extremely popular and was used extensively for sterility test isolators. The one complaint was the residual odour of acetic acid.

An experimental method of evaporation developed by the author was to introduce an aerosol of PAA into a wide diameter tube through which heated air flowed at a known rate. The subsequent PAA vapour was then directed into the isolator and circulated by use of distribution fans.

Hydrogen peroxide vapour generators

The most common method for producing hydrogen peroxide vapour has always been to evaporate hydrogen peroxide solution via a controlled delivery rate onto a heated plate at 100 to 110°C.

Filtered dry air blown across the heated plate entrains the vapour which is taken into and out of the isolator via hose connections, thus creating a closed loop for recirculation. The rate of delivery of the peroxide, the temperature used

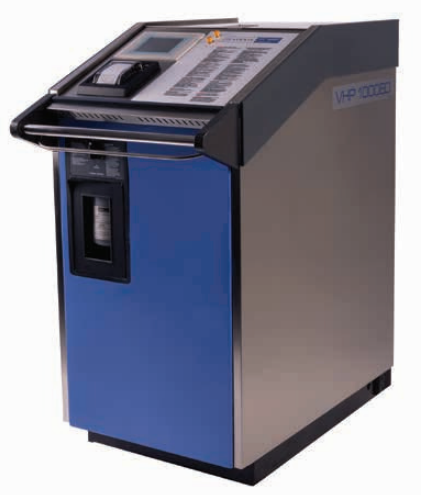

Figure 3: Steris VHP® gas generator

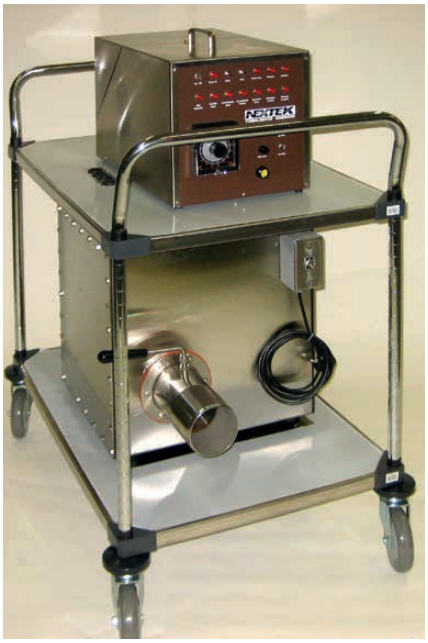

Figure 1: Nextec Model 1414RH formaldehyde gas generator/neutralizer with blower cart

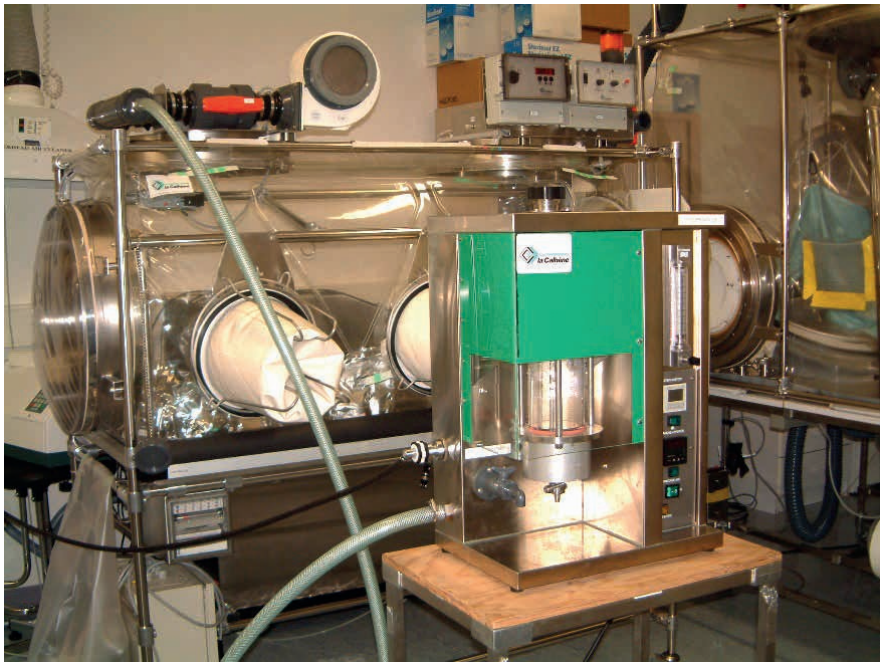

Figure 2: A La Calhene MAN 230 unit connected to a flexible film isolator

to evaporate it, the air flow rate and other parameters of the process are recorded on a printed chart at regular intervals throughout the cycle. All of these parameters can be calibrated and validated and are reproducible. This was the basis of the Steris VHP® gas generator is depicted in Figure 3.

This original unit used a container of hydrogen peroxide (approximately 1 litre of 35% electronic grade) supplied by Steris with a dedicated head that fitted into the machine. Unlike PAA evaporation (as described above) water and peroxide were simultaneously evaporated and blown into the isolator. As this increased the relative humidity (RH) inside the isolator a dehumidification phase had to be included in the process cycle.

The Steris VHP® gas generator has a four phase cycle: dehumidification, conditioning, exposure (sterilising) and aeration as part of the overall cycle. The device can be set to achieve a 10, 20 or 30% RH in the isolator prior to the start of the evaporation cycle. The evaporation process takes place over two phases: the conditioning phase, where the injection rate is such as to rapidly increase the vapour concentration in the isolator, followed by the exposure phase where the injection rate is adjusted to maintain the high concentration achieved. During this time the peroxide vapour returning to the generator (via the closed loop) passes through a catalytic molecular sieve that breaks the peroxide down into oxygen and water vapour, leaving the carrier air to continue through a dessicant before re-entering the evaporation chamber for fresh peroxide. The same process occurs during the aeration phase except that no peroxide is evaporated. The dessicant requires regeneration after 18 or more hours use.

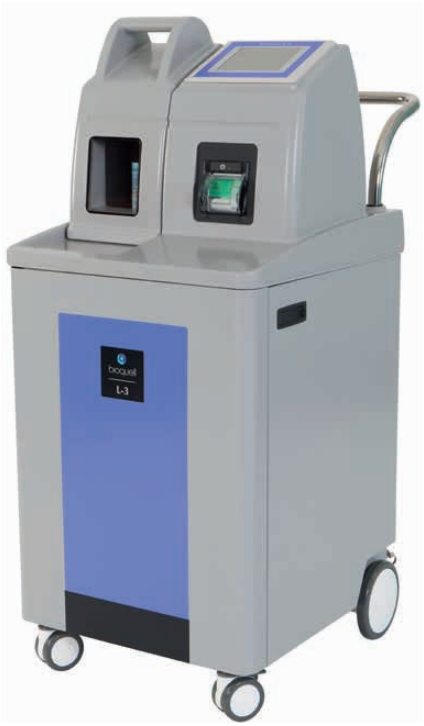

Figure 4: Bioquell L3 gas generator

The Steris VHP® gas generator was also adapted to be attached to a larger reservoir of peroxide to allow for longer, repeating conditioning and exposure phases. One early example was a sterilising tunnel for treating syringe tubs prior to entry into a sterile filling isolator so that a continuous run of the tunnel (up to 8 hours) could be achieved. Two generators were used so that as one was running the other was being recharged, i.e. the desiccant being dried.

The original Steris gas generator was ideal for sterility test or pharmacy isolators but later, with the increasing complexity and volumes of processing isolators, Steris also produced gassing modules that could be built into the isolator or attached to it and these units had a higher rate of evaporating the peroxide.

These devices were also adapted to be attached to a larger reservoir of peroxide to allow for longer, repeating conditioning and exposure phases.

One early example was a sterilising tunnel for treating syringe tubs prior to entry into a sterile filling isolator enabling an almost continuous supply of the syringe "nests" to the filling head.

Steris also produced a system for the sterilisation of freeze dryers using hydrogen peroxide vapour.

Another manufacturer of hydrogen peroxide vapour generators is Bioquell. Their gas generators (see Figure 4) come in a range of module sizes to suit the volume of the space to be decontaminated. Further larger units were developed for treating clean rooms and hospital wards, theatres, etc. A novel device, developed by Bioquell, was a rotating nozzle, fitted inside the isolator, which facilitated the distribution of the vapour. These machines also include a peroxide sensor that shows the gas concentration.

Later developments saw the dehumidification section taken over by the isolator system as these units became larger and larger. This entailed a separate dehumidification section added onto the isolator air handling system. Also the final aeration phase for the gassing cycle was conducted using the air handling system of the isolator. Both these changes shortened the sterilising cycle time as the original gas generators could not effectively dehumidify or aerate fast enough due to the volume of the isolator.

A Pioneer of the in-built or integrated peroxide gassing system was SKAN in Switzerland. Having developed a small model for decontaminating sterility test isolators, SKAN designed a larger model that was built into the air handling system of a large aseptic filling isolator in the UK.

A later development was by Metall+Plastic where the same built-in principle was employed but with an improved gas entrainment system into the isolator for better distribution.

In both these systems the gas was recirculated through the isolator and the filters during the exposure phase, ensuring that the entire body of the isolator and the associated air handling filter system was sterilised.

Getinge La Calhene introduced their version of the integrated peroxide gas generator named Steritrace.

Generally it was accepted that, in order to prevent undue condensation in the isolator, the internal temperature should be raised above ambient, for example 40 to 50°C, prior to the conditioning and exposure phases.

Current developments in the technology appear to indicate that micro-condensation of the peroxide takes place on exposed surfaces before any visible condensation is seen and that in this form the concentration of the peroxide is very high (> 50%). It is proposed that this is the reason for the very low concentrations of peroxide vapour that are needed to exert a sterilising effect at an air concentration of around 300 to 600 parts per million[6].

In the 1990s the author, using the Steris VHP® system, found this phenomenon in observing that, once a concentration of 250 to 300 ppm was recorded on peroxide measurement sensors, a series of duplicate biological indicators (in a 5 cubic meter isolator), pulled at 2 minute intervals from the start of the cycle, were rendered sterile.

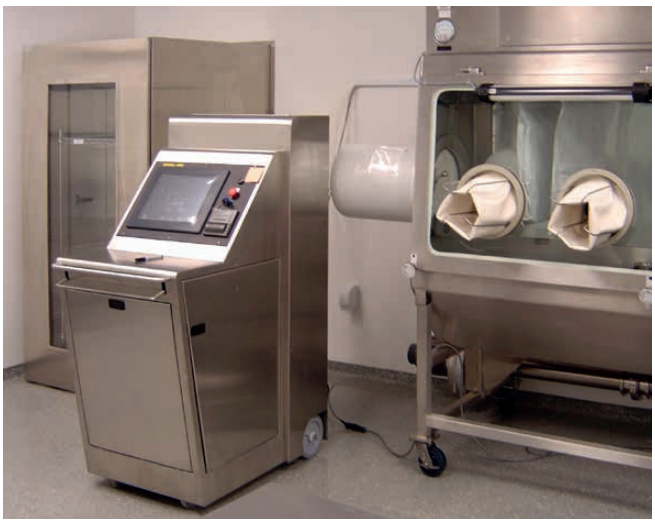

Figure 5: ClorDiSys CLORIDOX-GMP™ Gas Generator

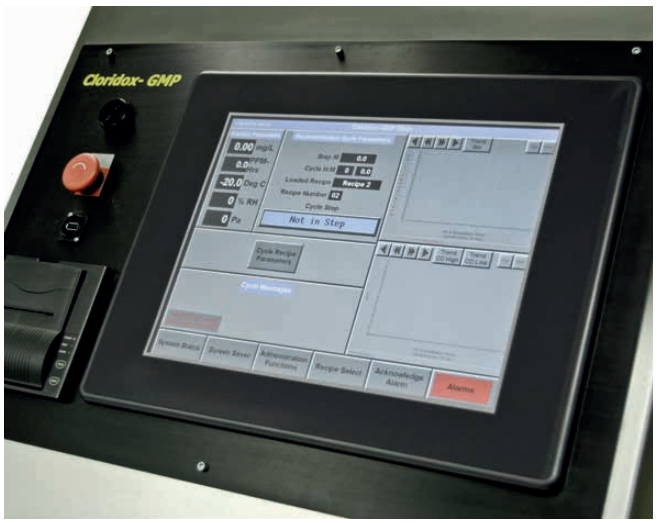

Figure 6: ClorDiSys CLORIDOX-GMP™ Gas Generator control panel

Chlorine dioxide gas generators

ClorDiSys Inc. produces a number of gas generators based on passing chlorine over sodium chlorite (in a cartridge) depending on the volume of space to be sterilised. The gas concentration is measured and controlled by an in-built monitor. Their CLORIDOX-GMP™ model is shown in Figures 5 and 6.

This agent and associated equipment has proved very popular in the germ free animal area and has also been used for the decontamination of rooms. The gas penetrates easily and importantly is removed very quickly which speeds up the sterilisation process.

Because it is based on a chlorine derived source it has not been used as much as the other sterilising agents due to some apprehension of toxicity and corrosion, but current reports on its use and also efficacy are encouraging.

In the final section of the history of isolator and containment technology a review of the validation practices in relation to sterility assurance will be presented.

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