Remington: The Science and Practice of Pharmacy

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Sterilization

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The aim of a sterilization process is to destroy or eliminate microorganisms which are present on or in an object or preparation, to make sure that this has been achieved with an extremely high level of probability and to assure that the object or preparation is free from infection hazards. The currently accepted performance target for a sterilization process is that it provide for a probability of finding a nonsterile unit of less than one in one million. That is, the process (including production, storage, shipment, etc) will provide a *Sterility Assurance Level* (SAL) equal to or better than 10^{-6}.

The variety and amounts of sterile products and their packages required for health care have increased continuously and been modified in recent years. Accordingly, sterilization technologies have adapted to the changing need. Some of these also are brought about by changing requirements and guidelines issued by regulatory or advisory bodies.

Not many years ago, sterility testing of the finished product was the basic means of monitoring the success of a sterilization process. Today, qualification and validation of the equipment and the process carried out in that equipment is considered essential. This stems from the general principles of Total Quality systems. National and international standards that define this system (ISO9000, EN29000, etc) indeed state that "...Sterilization is a *special* process because its efficacy cannot be verified by simple inspection and testing on the final product. ... For this reason, sterilization processes have to be validated before use, the performance monitored routinely and the equipment regularly maintained. ..."

The purpose of this chapter is to provide a basic understanding of the following sterilization methods currently being used in pharmaceutical technology and the equipment employed to carry out these methods:

Method	*Equipment*
Moist heat sterilization	Saturated steam autoclaves
	Superheated water autoclaves
	Air over steam autoclaves
Dry heat sterilization	Batch sterilizers
	Continuous tunnel sterilizers
Chemical "Cold" sterilization	Ethylene oxide
	Vaporized hydrogen peroxide
	Hydrogen peroxide/steam
	Other gases
Radiation sterilization	Electromagnetic
	Particulate
Filtration	Membranes

Definitions

The following terms, relating to sterilization, should be understood by those carrying out sterilization processes or handling sterile products:

Antiseptic—A substance that arrests or prevents the growth of microorganisms by inhibiting their activity without necessarily destroying them.

Aseptic Processing—Those operations performed between the sterilization of an object or preparation and the final sealing of its package. These operations are, by definition, carried out in the complete absence of microorganisms.

Bactericide—Any agent which destroys microorganisms.

Bacteriostat—Any agent which arrests or retards the growth of microorganisms.

Bioburden—The number of viable microorganisms in or on an object or preparation entering a sterilization step (usually expressed in colony forming units per unit of volume).

Disinfection—A process which decreases the probability of infection by destroying vegetative microorganisms, but not ordinarily bacterial spores. The term usually is applied to the use of chemical agents on inanimate objects.

Germicide—An agent which destroys microorganisms, but not necessarily bacterial spores.

Sterility—The absence of viable microorganisms.

Sterility Assurance Level (SAL)—A term related to the probability of finding a nonsterile unit following a sterilization step. It usually is expressed in terms of the negative power of 10 (ie, one in one million = 10^{-6}).

Sterilization—A process by which all viable microorganisms are removed or destroyed, based on a probability function.

Terminal Sterilization—A process which destroys all viable microorganisms within the final, sealed package.

Validation—The act of verifying that a procedure is capable of producing the intended result under all expected circumstances. This usually is accomplished through appropriate challenge(s).

Viricide—An agent which will destroy viruses.

Sterility as a Total System

It is necessary to reiterate the concept already briefly addressed in the introduction. The task of the technology we are dealing with is to provide the product in sterile conditions to the end user.

It is currently acknowledged that the *quality* of the product must be "built into" the process. This concept is particularly true when one of the essential qualities of the product is sterility.

Accordingly, the above-mentioned task is accomplished with a series of design, production and distribution steps that can be summarized as activities for the selection and routine checking of the following items:

Active constituents, additives, raw materials in general
Water used both as solvent and as washing/rinsing agent
Packaging suitable for the product and for the sterilization process that will be used
Working environment and equipment
Personnel

These procedures clearly have the purpose of providing the sterilization process with a product that has a minimum, definite and consistent bioburden. There are also the following activities:

Selection of the sterilization method that most suits the unit formed by the product and its packaging, and definition of the process variables for obtaining the intended SAL
Selection of the machine that is most suitable for performing the selected method and of the utilities that this machine requires
Qualification and validation of the machine and of the process
Routine checking of the process
Checking of the results of the sterilization process
Proper storage of sterile goods and verification that their sterility is maintained with full reliability throughout the allowed storage period
Delivering, opening and using sterile goods without recontamination.

It also should be noted that, on October 11, 1991, the FDA proposed new regulations for aseptic processing and terminal sterilization. The proposed rules requires manufacturers of sterile products to use terminal sterilization wherever possible. The proposal will affect 21 CFR 211, 314 and 514. Aseptic processing may be used only in those cases where terminal sterilization has significant detrimental effects on the product. This ruling is based on the ability to prove higher SAL's with current terminal sterilization processes, thus reducing the risk of a nonsterile unit reaching the patient.

Contamination

Certain facts about microorganisms must be kept in mind when preparing sterile products. Some microbes (bacteria, molds, etc) multiply in the refrigerator, others at temperatures as high as 60°. Microbes vary in their oxygen requirements from the strict anaerobes that cannot tolerate oxygen to aerobes that demand it. Slightly alkaline growth media will support the multiplication of many microorganisms while others flourish in acidic environments. Some microorganisms have the ability to utilize nitrogen and carbon dioxide from the air and thus can actually multiply in distilled water. In general, however, most pathogenic bacteria have rather selective cultural requirements, with optimum temperatures of 30 to 37° and a pH of 7.0. Contaminating yeasts and molds can develop readily in glucose and other sugar solutions.

Actively growing microbes are, for the most part, vegetative forms with little resistance to heat and disinfectants. However, some forms of bacteria—among them the bacteria that cause anthrax, tetanus and gas gangrene—have the ability to assume a spore state, which is very resistant to heat as well as to many disinfectants. For this reason, an excellent measure of successful sterilization is whether the highly resistant spore forms of nonpathogenic bacteria have been killed.

The nature of expected contamination and the bioburden are important to the pharmacist preparing materials to be sterilized. The raw materials he works with rarely will be sterile and improper storage may increase the microbial content. Because the pharmacist seldom handles all raw materials in a sterile or protected environment, the environmental elements of the manufacturing area (air, surfaces, water, etc) can be expected to contribute to the contamination of a preparation. The container or packaging material may or may not be presterilized and thus may contribute to the total microbial load.

Understanding the nature of contaminants prior to sterilization and application of methods for minimizing such contamination is vital to preparing for successful pharmaceutical sterilization. Examples of such methods include:

Maintenance of a hygienic laboratory.
Frequent disinfection of floors and surfaces.
Minimization of traffic in and out of the area.
Refrigerated storage of raw materials and preparations which support microbial growth.
Use of laminar airflow devices (see page to be referenced) for certain critical operations.
Use of water that is of appropriate USP quality and is free of microbial contamination. It is preferrable to use presterilized water to avoid any possible contamination.

Methods

General

The procedure to be used for sterilizing a drug, a pharmaceutical preparation or a medical device is determined to a large extent by the nature of the product. It is important to remember that the same sterilization technique cannot be applied universally because the unique properties of some materials may result in their destruction or modification. Methods of inactivating microorganisms may be classified as either physical or chemical. Physical methods include moist heat, dry heat and irradiation. Sterile filtration is another process, but it only removes, not inactivates, microorganisms. Chemical methods include the use of either gaseous or liquid sterilants. Guidelines for the use of many types of industrial and hospital sterilization are available.[1-10]

Each sterilization method can be evaluated using experimentally derived values representing the general inactivation rates of the process. For example, a death rate or survival curve for a standardized species can be diagrammed for different sterilization conditions. This is done by plotting the logarithm of surviving organisms against time of exposure to the sterilization method. In most instances, these data show a linear relationship, typical of first-order kinetics and suggest that a constant proportion of a contaminant population is inactivated in any given time interval. Based on such inactivation curves, it is possible to derive values that represent the general inactivation rates of the process. For example, based on such data, it has become common to derive a decimal reduction time or D value, which represents the time under a stated set of sterilization exposure conditions required to reduce a surviving microbial population by a factor of 90%.

D values, or other expressions of sterilization process rates, provide a means of establishing dependable sterilization cycles. Obviously, the initial microbial load on a product to be sterilized becomes an important consideration. Beyond this, however, kinetic data also can be used to provide a statistical basis for the success of sterilization cycles. A simple example will suffice (Fig 1). When the initial microbial contamination level is assumed to be 10^6, and if the D value of the sterilization process is 7 minutes, complete kill is approached by application of 6 D values (42 minutes). However, at this point reliable sterilization would not be assured because a few abnormally resistant members of the population may remain. In this example, by extending the process to include an additional 6 D values, most of the remaining population is inactivated, reducing the probability of one organism surviving to one in one million.

Moist Heat

Essentials of Steam Sterilization Kinetics—Let us suppose a system contaminated by microorganisms (which we assume, for the sake of simplicity, to be pure and homogeneous) is immersed in pressurized saturated steam, at constant temperature; for example a vial containing an aqueous suspension of a certain spore-forming microorganism.

It has been shown experimentally that, under the above conditions, the reaction of thermal degradation of the microorganism obeys the laws of chemical reactions: the rate of reduction of the number of microorganisms present in the system in each moment is proportional to the actual number itself. The proportionality coefficient is typical of the species and conditions of the chosen microorganism.

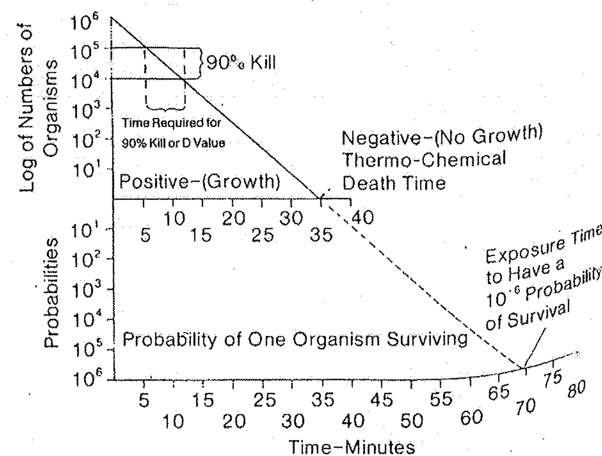


Fig 1. Sterilization model using D values.

The degradation reaction, (the sterilization process) therefore, develops like a first-order chemical reaction in which the reaction rate is proportional, in each moment, only to the amount of microorganisms still to be inactivated. This seems to be obvious for dry sterilization, but less rigorous for steam sterilization, in which the water vapor molecules also seem to take part in the reaction. Actually, this bimolecular reaction is of the first order, since the steam is present in high excess during the entire reaction and its concentration may be regarded as constant.

The most frequently used mathematical expression of the above facts is

$$N = N_0\, 10^{-t/D} \qquad (1)$$

where N_0 = initial number of microorganisms, t = elapsed exposure (= sterilization time), N = number of microorganisms after the exposure time t and D = "decimal decay time," defined as the time interval required, at a specified *constant* temperature, to reduce the microbial population being considered by 1/10 (ie, by one logarithmic value, eg, from 100% to 10% or from 10% to 1% of the initial value).

The D value is inversely proportional to the first-order reaction coefficient and is therefore typical of the species and conditions of the chosen microorganism. Depending on the initial hypothesis of exposure at constant temperature, each D value always refers to a specified temperature.

Equation 1 allows one to draw a first very important conclusion: the time required to reduce the microorganism concentration to any preset value is the function of the initial concentration.

The sterilization reaction is therefore neither an "all-or-nothing" process nor a "potential barrier" process as was once thought.

It also is evident immediately that the effect of sterilization at the same constant temperature will be very different depending on the D value of the contaminating microbial species (or on the largest D value in the usual case of mixed contamination). Figure 2 shows that the same reduction ratio for different species is achieved after exposure time proportional to the D value of each species. The graph derives only from Eq 1 and from the definition of D value. The basic hypothesis of the temperature being constant is thoroughly valid.

Sterility Is a "Probable" Effect of Exposure Time—Let us now consider what happens within a batch of units (vials, bottles or others) with an initial constant unit contamination of 100 microorganisms = 10^2. If the D value at 121° is assumed = 1, after 1 minute at 121°, the reduction = to 10^1 = 10 microorganisms is achieved; after another minute, only 10^0 = 1 microorganism is still surviving. After another minute the surviving microbial population would be 10^{-1} = 1/10 microorganism. A contamination of 1/10 must not be understood to mean that each unit contains 1/10 of a microorganism, which is biologically meaningless (in this case the unit probably would be sterile. . .) but that there is a probability of having 1/10 of the units still contaminated within the batch of sterilized units.

In fact, 3 minutes would be the necessary time to reduce the microbial population to a single surviving microorganism if the initial population were ten times larger than the one at issue. This higher initial contamination could be regarded either as a ten times larger number of microorganisms in the same unit, or as the initial contamination of a ten times larger unit.

If the unit is not considered any longer as the single vial or bottle, but as the whole of all the items produced over a period of time, the initial number of microorganisms present in each item has to be multiplied times the number of items produced, and the exposure time to achieve the reduction to the same number of viable microorganisms left in the whole of the items produced, has to be increased correspondingly. The following example will be helpful to focus the matter.

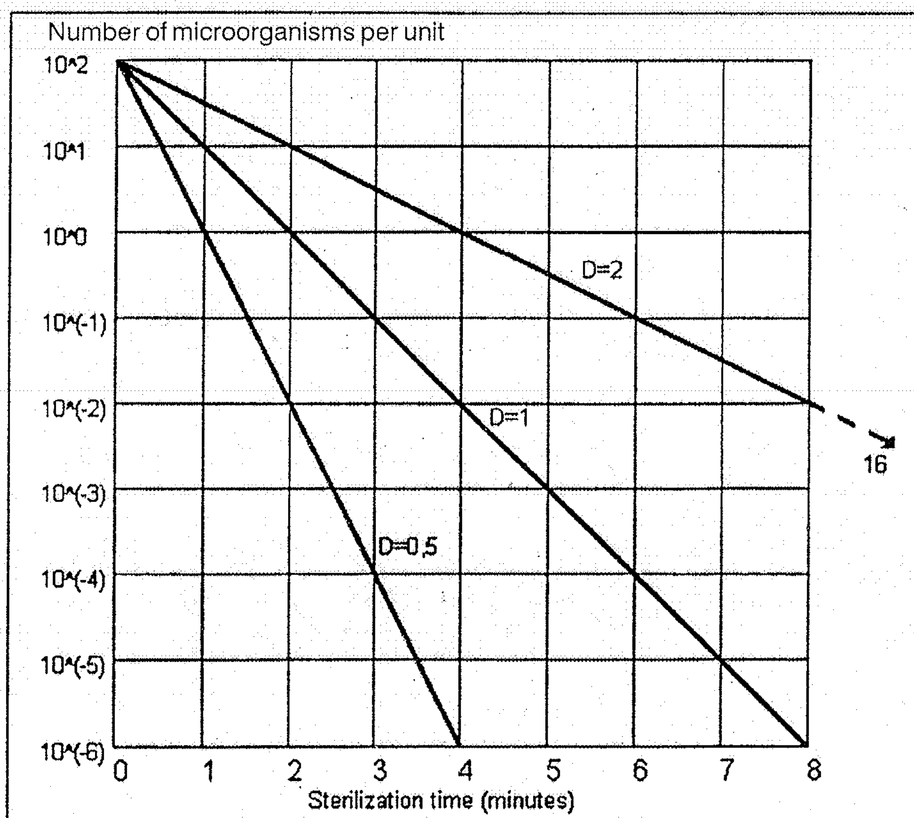


Fig 2. Effect of varying D values on sterilization rate (courtesy, Fedegari Autoclavi).

A new sterile product in ampules has to be manufactured; the number of ampules to be produced over all the life period of the product is expected to be 10^{10}. The maximum number of contaminated ampules deemed to be acceptable is $10^{\circ} = 1$: this obviously means that the probability of having nonsterile ampules after sterilization must not exceed 10^{-10}. Let us also suppose that the microbial population within each ampule after the filling and the sealing does not exceed 10^3 microorganisms. These must be destroyed by means of moist heat-terminal sterilization at 121°. The applicable D value is 1 minute. The total number of microorganisms to be destroyed during the life of the product will be

$$10^{10+3} = 10^{13}$$

If this whole microbial population were exposed to moist heat at 121° over a period of 13 minutes, it would be reduced to 10^{-13} times it initial number, ie, to $10^{13-13} = 10^{\circ} = 1$. The exposure time of 13 minutes thus would be sufficient (under all the other above hypotheses) to prevent the total number of contaminated ampules from exceeding the value of one.

From the point of view of each single ampule, 13 minutes of exposure would reduce the microbial population to the theoretical value of

$$10^{3-13} = 10^{-10}$$

To interpret this numeric value as the probability of still having one contaminated ampule in ten billion sterilized ampules means that a single ampule will still be contaminated out of a whole lot of 10^{10}. This probability value is defined as PNSU (Probability of Non Sterile Unit).

In recent times the PNSU as a sterility evaluation criterion is being replaced by the SAL (Sterility Assurance Level). The name itself could generate some misunderstanding since a level of assurance commonly is deemed to be good if high, but SAL seems to have been defined in such a way that its numerical value is the same as PNSU. This notwithstanding, it is sometimes calculated as the reciprocal value of PNSU. The SAP (Sterility Assurance Probability) criterion has been proposed as well and SAP seems for the moment to have been granted the same definition of PNSU, even if it would be better understandable if its value approached unity after a satisfactory sterilization.

The above discussion and example lead to the conclusion that the optimum exposure time for a sterilization process must take into account not only the initial microbial population within the single item to be sterilized and the species and conditions of the contaminating microorganism, but also the total number of items expected to be sterilized over the life of the product.

Effect of Temperature Changes—All the above considerations have been developed under the basic assumption that the temperature is kept constant during the entire exposure time. It seems rather obvious that the D value will change as the temperature changes. If the D values experimentally obtained for a given microbial species are plotted on a semilogarithmic chart as the function of the temperature T, a path similar to Fig 3 is obtained.

In this case, it can be seen that D value is 1 minute at 121° (ie, the average value which very often is assumed to be acceptable in the absence of more exact experimental data). It also can be seen that D value varies by a factor of 10 if the temperature varies by 10°.

The z value is defined as the temperature coefficient of microbial destruction, ie, as the number of degrees of temperature which causes a 10-fold variation of D (or, more generally, of the sterilization rate).

The z-values generally oscillate between 6 and 13 for steam sterilization in the range 100 to 130° and z value often is assumed to be equal to 10 in the absence of more precise experimental data.

The fact that D value varies by 10 times for a variation of 10° when z = 10 must not lead to the false assumption that D varies by one time (ie, doubles) for an increase of 1°. Obviously, this is not true.

It is actually a matter of finding the number which yields 10 when raised to the tenth power. This number is 1.24. Therefore, a variation of 1° entails a variation of D value of 24%.

This is quite a significant number, which illustrates the dramatic effects which are generated when the sterilization

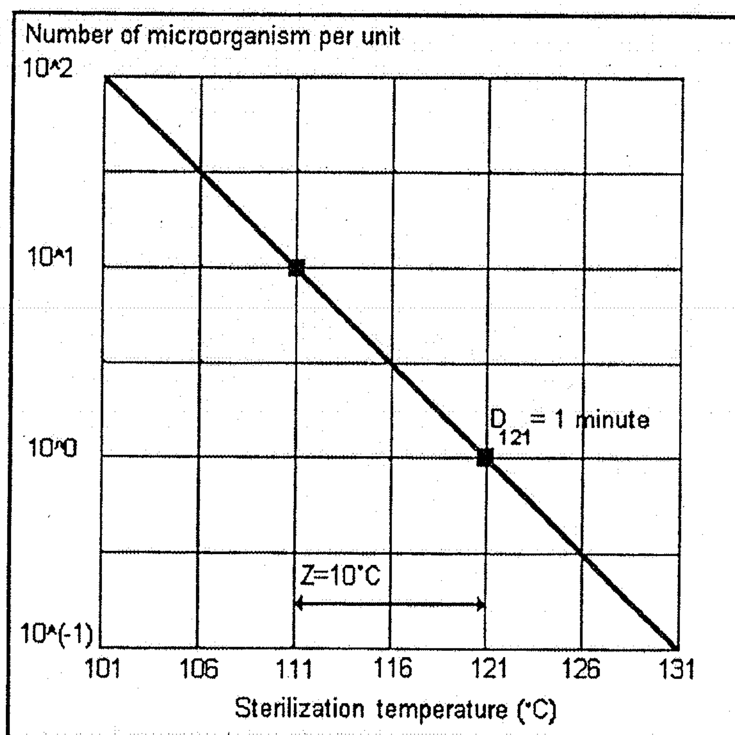


Fig 3. Effect of temperature on microbial destruction (courtesy, Fedegari Autoclavi).

temperature is also only a few degrees lower than the expected value, perhaps only in some areas of the sterilizer load.

It is also useful to remember that the effect of temperature variation decreases considerably as the temperature rises and drops to approximately 1/2 (or even less) for dry sterilization at approximately 200°. Under these condition the z value is about 20 instead of about 10. Therefore, the small temperature differences which can be so dramatic in steam sterilization have much less effect in dry sterilization.

The foregoing refers to average values since the actual D values and z values depend to a large extent on the medium which contains the microorganisms and on their history. At 121° no microorganism has exactly D = 1 and z = 10. However, the combined use of these two parameters in calculating F_0 and PNSU provides ample margins of safety with regard to the microorganisms with which we deal commonly.

F_0 or Equivalent Sterilization Time at 121°—It is of the utmost interest to calculate the lethal effect of the exposure of a microbial population to a variable temperature, T, by relating it to an hypothetical sterilization performed at a constant temperature, T_0, for the time, t_0. If the constant reference temperature is assumed equal to 121.1° (originally 250°F) and the z value equal to 10, the equivalent time is termed F_0.

Thus, F_0 is the equivalent exposure time at 121.1° of the actual exposure time at a variable temperature, calculated for an ideal microorganism with a temperature coefficient of destruction equal to 10.

First introduced in the *Laboratory Manual for Food Canners and Processors* by the National Canners Association in 1968, F_0 has become a common term in pharmaceutical production since the FDA used it extensively in the "Proposed rules" of June 1st, 1976 (21 CFR 212.3) with the following meaning:

> F_0 means the equivalent amount of time, in minutes at 121.1°C or 250°F, which has been delivered to a product by the sterilization process.

For the calculation of it

> A z value of 10°C or 18°F is assumed; the term z value means the slope of the thermal death time curve and may be expressed as the number of degrees . . . required to bring about a tenfold change in the death rate.

In practice, the knowledge of the temperature values as the continuous function of elapsing time is not available, and F_0 is calculated as

$$F_0 = \Delta t \, \Sigma \, 10^{\frac{T-121.1}{z}} \quad (2)$$

where Δt = time interval between to following measurements of T, T = temperature of the sterilized product at time t and z = temperature coefficient, assumed to be equal to 10.

Saturated Steam

Principles—Sterilization with saturated steam is the method that provides the best combination of flexibility in operation, safe results and low plant and running costs.

The sterilizing medium obviously is pressurized saturated steam and the typical operating temperature is 121° (250°F), but higher or lower temperatures often are used.

The term *dry* saturated steam sometimes is used: it should be made clear that this is an "ideal" condition of steam, and that *moist* saturated steam is used in practice for sterilization. However, the steam must entrain the smallest possible amount of condensate. The "water vapor ratio" of the steam defines the amount of condensate entrained by 100 parts by weight of moist steam; a water vapor ratio of 0.95 means that 100 g of steam consist of 95 g of dry saturated steam plus 5 g of condensate which is, or should be, *at the same temperature as the steam.*

The reliability of sterilization performed with saturated steam is based on three particular characteristics of this medium.

When steam condenses, it releases calories at a *constant temperature* and in a *considerable amount:* 1 kg of pure saturated steam condensing at 121° (turning into water at 121°, thus without cooling) releases as much as 525 kcal.

The temperatures and pressures of saturated steam have a two-way correlation. Once the temperature of the steam is determined, so is its pressure, and vice versa. Saturated steam at 121° inevitably has a pressure of 2.05 abs bar.

This entails two very interesting practical possibilities: (1) a pure saturated steam autoclave can be controlled indifferently according to the temperature parameter or according to the pressure parameter and (2) regardless of the parameter used for control, the second parameter can be used easily to cross-monitor the first one.

One gram molecule of water (18 g, ie, 18 mL in the liquid state) as steam at 121° and 2.05 abs bar occupies a volume of approximately 15 L. This means that when steam condenses at 121° it shrinks in volume by almost 1000 times. Accordingly, additional available steam *spontaneously* reaches the object to be sterilized. The condensate that forms can be removed easily from the autoclave chamber by means of a condensate discharge or, with a more modern technique, by continuous and forced bleeding (as occurs for example in so-called *dynamic steam* sterilizers).

However, three other phenomena must be considered.

In order to perform its microorganism inactivating action (coagulation of cellular proteins), the steam, or more generally the moist heat, must make contact with the microorganisms. This can occur directly or indirectly. For example, it occurs directly when the steam that is present in the autoclave chamber is in direct contact with a surgical instrument. It instead occurs indirectly when moist steam is generated (by heat exchange with the steam present in the chamber) inside a sealed ampul that contains an aqueous solution. However, it is evident that it is not possible to steam-sterilize the inside of an empty closed ampul or the contents of an ampul if they are constituted by an anhydrous oil-based solution.

The air that is initially present in the autoclave chamber and the *incondensables* that possibly are entrained by the steam (generally CO_2) have molecular weights, and thus densities, 1.5 to 2 times higher than steam (under equal temperature/pressure conditions). Therefore, the air must be eliminated initially from the chamber and the steam must not introduce incondensables in the chamber; otherwise, these tend to stratify in the lower portions of the chamber, creating intolerable temperature gradients.

When closed nondeformable containers that contain aqueous solutions are sterilized, the pressures inside them can reach values far above those of the chamber. All air has been removed from the chamber, which in fact only contains steam: accordingly, at 121° the pressure is 2.05 abs bar. The container instead almost always has a head space that contains air (or other gases).

During sterilization, the aqueous solution of the container produces a vapor pressure which is approximately equal to 2.05 abs bar, but this value is increased by the partial pressure of the air of the head space; assuming that its initial value is 1.0 bar, it will increase to approximately 1.3 bar due to heating.

Pressure increases also will occur due to the thermal expansion of the solution (which is not entirely compensated by the expansion of the glass of the container) and because any gases dissolved in the solution may leave it.

Generally, in the conditions described above the total pressure inside the container exceeds by approximately 1.4 bar the pressure in the chamber if the initial head space is, as usually occurs, 10 to 20% of the total volume of the container. This overpressure generally is well tolerated by glass ampules, even those of considerable capacity (20 to 30 mL). However, it becomes hazardous for glass containers fitted with rubber stoppers held in place by a seal (due to the risk of stopper lifting) and intolerable for deformable containers, such as rigid (and even flexible) plastic containers, prefilled syringes or cans. In all these cases, it is necessary or convenient to use the *counterpressure* sterilization methods (described later).

Saturated Steam Autoclaves—

Materials—All autoclaves intended for the pharmaceutical industry are made of Class AISI 316 stainless steel, including valves and piping. See Fig 4. Only the service elements arranged *downstream* of the autoclave (for example the vacuum pump or the condensate discharge) are accepted if they are made of other materials. The service elements *upstream* of the autoclave (eg, heat exchangers or water pumps) also must be made of stainless steel.

Silicone rubber or Teflon and derivatives thereof generally are used for the gaskets (of doors, valves, etc).

Structure—Saturated steam autoclaves generally have a quadrangular, or rarely cylindrical, chamber. The doors are generally quadrangular even if the structure is cylindrical; in

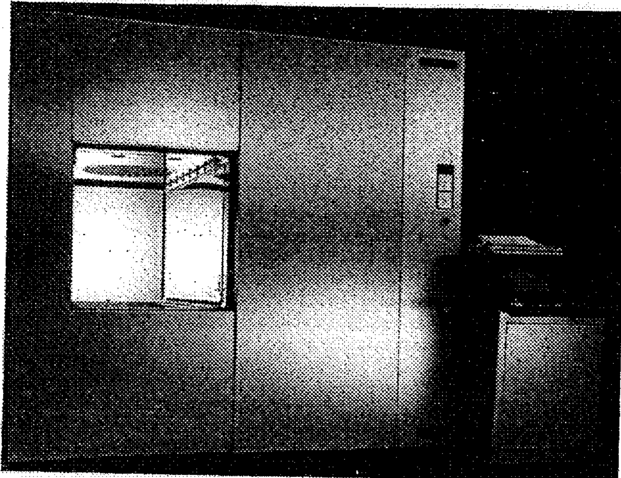


Fig 4. A modern computerized steam autoclave with horizontal sliding door (courtesy, Fedegari Autoclavi).

this case, the doors are inscribed in the circumference. There may be one or two doors: when the autoclave leads to a sterile room, there are always two doors.

Two-door autoclaves often are used when this requirement does not occur but the need is nonetheless felt to separate the loading area, where products *to be sterilized* are placed, from the unloading area, where *already sterilized* products are placed. *This concept applies to all types of sterilizers.*

Doors may be of various kinds. The most common types are

Hinged, manually operated, retained by radial locking bars, with a solid and fixed gasket.

Hinged, semiautomatically operated, retained by means of abutments in which the door engages automatically and with a movable gasket activated by compressed air.

Vertically or laterally sliding, with retention and gaskets as mentioned immediately above.

Saturated steam autoclaves generally are jacketed. There is no room here to discuss the various kinds of jacket and their purposes. However, there are two ways to feed steam into the jacket and into the chamber:

Single Feed—the steam circulates first in the jacket and passes from the jacket into the chamber.

Separate Feed—usually the chamber is fed pure steam and the jacket is fed industrial steam.

Single feed steam has some advantages in terms of control, but separate feed steam is preferred since it provides better assurances of lack of microbiological and particle contamination.

Management Systems—The management systems used on currently manufactured autoclaves are programmable logic controllers (PLCs) or personal computers (PCs) or, sometimes, combinations of PLCs and PCs. This is also true for other kinds of autoclaves and sterilizers, which we will discuss later.

However, a very large number of autoclaves controlled by electropneumatic systems are still in operation and still perform acceptable work. Naturally, the current control systems offer a kind of performance that was undreamed of earlier.

Pressure or temperature control (we mentioned previously that these parameters are interchangeable for a saturated steam autoclave) generally is performed with a proportional-integral-derivative method. Control by temperature is the generally accepted scheme since it is not influenced by trapped air. Sterilization can be time-managed or F_0-managed (with the F_0 being accumulated by heat probes enabled for this function), or time-managed with simultaneous calculation of F_0 for monitoring purposes.

Some management systems offer exceptional flexibility in composing programs and in setting parameters even to operators that have no knowledge of electronic programming. The information provided in real time (on same display device) is extremely detailed; so is the permanent information, which can be produced on paper or stored on various kinds of electronic medium.

Process—

Initial Removal of the Air from the Chamber—The main reason the air must be removed from the autoclave chamber has been pointed out above.

Loads often are made up of porous materials or materials packaged in sterilization paper or in plastic/paper bags, or contained in filter boxes. All these situations require reliable and rapid removal of the air from the load. The so-called *gravity* removal method is considered obsolete. Modern autoclaves have a water-ring vacuum pump that can produce a vacuum of approximately 70 residual mbar in the chamber. Accordingly, only about 10% of the air remains in the chamber. There are essentially two methods for completing air removal:

Pulsed Vacuum—Once the initial vacuum has been reached, the pump is stopped and steam is introduced in the chamber (up to approximately atmospheric pressure), then vacuum is produced again. Three or more of these vacuum/steam pulses are performed.

Dynamic Vacuum—Once the initial vacuum has been reached, the pump continues to run, but at the same time a 5- to 10-minute injection of steam is performed (from the side of the chamber that lies opposite the vacuum drain).

Modern autoclaves are capable of performing either of these methods, chosen according to the load to be processed.

Heating-Sterilization—During heating phases, and much less during the sterilization phase, considerable amounts of condensate form in the chamber. Except for particular instances, this condensate must be removed from the chamber. There are basically two extraction methods:

A condensate trap located at the bottom of the chamber. This is the simplest and cheapest method, but it causes significant pressure drops, and therefore temperature drops, inside the chamber due to the inertia of the condensate trap. Essentially, it discharges not only the condensate but also significant amounts of steam, which cause instantaneous expansion, and thus cooling, of the steam that remains in the chamber.

Dynamic steam. This is the most reliable and elegant system, but is also more expensive. During the heating and sterilization phases, the vacuum pump is kept running and draws from the chamber all the condensate that forms in it through a low-capacity valve. A certain amount of steam is naturally aspirated continuously, and a *dynamic* condition of the steam is thus produced, hence the name of the method.

Autoclaves also are required to have a continuous steam bleed past the controlling sensor in the drain line.

Post-Sterilization Phases—These may be different according to the material to be sterilized and depending on the results to be obtained on the material itself. The most common solutions are

1. **Vacuum and Time-Controlled Vacuum Maintenance**—This method is used to dry and simultaneously cool loads of solid materials, both porous and non-porous. It is performed by restarting the vacuum pump until a preset value (eg, 100 mbar) is reached; the pump then is kept running for a preset time (eg, 20 minutes).

2. **Cooling by Circulating Cold Water in the Jacket**—This method is used to cool containers that are partially filled with solution (eg, culture media) and closed with sleeve (Bellco-type) stoppers. Naturally, with these loads Item 1 is not applicable, since the solution would boil, and Item 3 is dangerous due to possible contaminations. This method is performed by removing the steam present in the chamber through the introduction of compressed sterile air at a pressure that is equal to, or greater than, the sterilization pressure. Then, cold water is circulated in the jacket. The pressurized compressed air in the chamber has two purposes: (1) to prevent the solution from boiling and (2) to improve heat exchange between the load and the jacket.

3. **Cooling by Spraying Water on the Load**—This method generally is used for loads of filled and closed ampuls and plastic intravenous containers. It is performed with deionized water (to avoid salt residues on the ampuls) which is nebulized onto the load by means of a sparger provided in the ceiling of the chamber. Naturally the ampuls, which preferably are arranged in an orderly fashion, must be contained in trays with a perforated bottom. Nebulization of the water causes a rapid con-

densation of the steam that produces a sudden pressure drop in the chamber, whereas the pressure inside the ampuls still remains rather high because the solution cools rather slowly. Ampuls of good quality (even large ones up to approximately 20 mL) tolerate this method adequately. Cooling stops when the solution inside the ampuls has reached the temperature of 70 to 80°. In this manner, the load, removed from the autoclave, still contains enough heat energy to dry spontaneously.

4. **Ampul Tightness with Fast Vacuum**—The pressure stress described in Item 3, above, is produced deliberately and increased by activating the vacuum pump as soon as the sterilization phase ends. The pressure in the chamber quickly drops to values that can reach 150 to 200 mbar (obviously this value can be controlled easily), whereas the pressure inside the closed ampuls initially remains above 3.0 bar. The ΔP thus produced breaks ampuls with "closed defects," ie, thinner regions and tensions in the glass, closed cracks, etc.

Obviously, if the ampuls have "open defects" (ie, holes at the tip or open cracks), the ΔP does not arise or is very small and thus the ampuls rarely break. What happens instead is that the solution in the ampul boils and thus evaporates, reducing the volume of the solution. Unfortunately, this evaporation is very limited. Since it requires a considerable amount of energy, the solution cools very quickly and the boiling ends. One cannot rely on the transmission of heat from the adjacent ampuls or from the jacket, since the chamber is evacuated.

It is evident that in such conditions, solution in the liquid state leaks from the ampuls; at least from the "open defects" that lie *below* the level of the solution. Accordingly, it may be convenient to load the ampuls upside down (i.e. with their tip pointing downward) if it is known that most defects occur at the tip or shoulder of the ampules.

Naturally, the breakage of the ampuls or the leakage of solution soils the load, which must therefore be washed and dried. With appropriate methods it usually is possible to achieve all this in the autoclave itself.

5. **Cooling as in Item 3, but with Air Counterpressure**—In many cases it is not possible or reasonable to subject the load, during cooling, to the pressure stress that arises with the method described in Item 3. In such cases, it is possible to remove the steam present in the chamber by replacing it with sterile compressed air at a pressure which is equal to, or higher than, the sterilization pressure. Only after this has occurred does the cooling water spray described in Item 3 begin. This method only prevents the load from suffering the pressure stress of the cooling phase, whereas the stress of the sterilization phase is unavoidable. Reference is made to the section on "Counterpressure Methods" below for an explanation of this phenomenon and for the autoclaves that allow to avoid it.

6. **Spontaneous Cooling**—In some particular cases it may be necessary to resort to this cooling method, which is the simplest but also obviously requires a very long time. Clearly, at the end of this cooling the autoclave will be in vacuum, and the longer the cooling the deeper the vacuum.

7. **Ampul Tightness Test with Dye Solution Penetration**—This test generally is performed with an aqueous solution of methylene blue. However, it is also possible to use other dyes. This test is effective only on "open defects" of ampuls and is performed as follows:

a. Vacuum in the chamber to approximately 100 to 150 mbar.
b. The chamber is filled with the colored solution until the load is completely covered; the ampuls must of course be contained in appropriate trays that do not allow them to escape, since they tend to float.
c. During this filling operation, the chamber vacuum reached in Item a is maintained continuously by connecting the vacuum pump to the ceiling of the chamber
d. The colored solution is pressurized at 2 to 3 bar and is maintained in this condition for 30 to 60 minutes or more.
e. The colored solution is discharged and recovered.
f. The load is washed several times with spray water.
g. The load is washed by flooding the chamber.
h. The washing water is discharged.

There are alternatives to this method, such as electronic spark discharge inspection which detects leakage of liquid from the ampul by a decrease in resistance across electrodes placed across the ampul.

The vacuum is not maintained continuously while the chamber is being filled with the colored solution.
The vacuum is produced only after filling the chamber with the colored solution.
The vacuum is not produced at all.

This test has in any case the following problems:

It has been demonstrated extensively that with usual values for dye concentration, differential test pressure and test time, tip holes with a diameter of less than 5 to 10 µm allow very small amounts of colored solution to enter. This prevents detection of the coloring of the ampuls during subsequent checking.

The preparation of sterile colored solution for each test entails very high costs.

Recovery and reuse of the colored solution entails keeping it in conditions that prevent microbial proliferation (80°) and subjecting it to sterilizing filtration prior to each test. All these procedures are expensive and complicated. In any case, the solution recovered from each test is contaminated chemically by the broken or defective tested ampuls.

Decolorization/destruction of the solution is very difficult, since methylene blue is very stable; however, good decolorization results have been achieved by using ozone. The use of amber glass ampuls makes detection of the dye difficult.

Sterilizing the Air Introduced in the Chamber—In the previous paragraphs we noted that it is often necessary to introduce air in the chamber, especially in poststerilization phases. This air must be sterile, otherwise it may recontaminate the sterilized load and can, in any case, contaminate the sterile environment if the autoclave is of the two-door type connected to the sterile area.

The air generally is sterilized by filtration using a system that is part of the autoclave. It is thus necessary to

Provide a filtration cartridge with sterilizing porosity.
Allow *in situ* sterilization of the assembled filtration system with an appropriate sterilization program of the autoclave itself.
Ensure that the filtration system and the line for connecting it to the autoclave maintain their sterility between one production sterilization program of the autoclave and the next.
Allow validation of all of the above described procedures.

If one wishes to operate in perfect safety, the filtration system also should be subjected to an integrity test each time it is operated.

Counterpressure Methods

Autoclaves operating with counterpressure are defined as devices able to control, during sterilization, the pressure of the moist sterilizing medium independently of its temperature.

Conventional pure saturated steam autoclaves do not belong to this category. The temperature of the pure saturated steam present in the chamber in fact automatically generates a specific pressure which cannot be modified without modifying the temperature as well. If the temperature of the steam is 121°, its pressure is unavoidably 2.05 bar abs and vice versa, assuming no trapped air.

For many kinds of load it is necessary or convenient to use an autoclave operating with counterpressure.

In order to understand this need, let us see what happens in a conventional autoclave during the sterilization of a rigid container partially filled with an aqueous solution and closed tight. For the sake of simplicity, let us assume that the container is filled with pure water.

A glass bottle is filled partially in standard conditions: 20° and 1.013 bar; the bottle is closed with a rubber stopper and aluminum seal. In the head space there is a total pressure of 1.013 bar, which is actually the sum of two factors: a partial water-vapor pressure which corresponds to the vapor pressure of water at 20°, ie, 0.025 bar, and a partial air pressure of 0.988 bar.

When the bottle is subjected to the sterilization phase at 121°, these two factors change as follows:

	Initial condition		Sterilization condition
Partial water-vapor pressure	0.025	→	2.050 bar (1)
Partial air pressure	0.988	→	1.330 bar (2)
Total pressure in head space	1.013	→	3.380 bar abs

Value 1, 2.050 bar, is obviously the pressure of water vapor at 121° and *corresponds to the pressure which occurs in the autoclave chamber*. Value 2, 1.330 bar, is a theoretical value which is calculated by applying the law of perfect gases

to air, ie

$$0.988 \times \frac{121 + 273}{20 + 273} = 1.330$$

Therefore, the total pressure of 3.380 bar abs is also a theoretical value.

There are some reports which demonstrate that the *practical* value is slightly higher than the theoretical one and largely depends on the ratio between the head space and the volume of the filling solution. The practical pressure of the head volume is, on average, higher at 121° by approximately 1.40 bar, with respect to the pressure in the chamber. This is caused by two mechanisms:

The thermal expansion of water is significantly greater than that of glass and increases very rapidly as the temperature rises. The specific volumes of water at the temperatures we are interested in are in fact

Temperature °C	Specific volume mL/g
0	1.0002
4	1.0000 (maximum density)
20	1.0017
120	1.0606

In passing from 20 to 121°, water increases its volume by approximately 6% according to the following ratio:

$$\frac{1.0606}{1.0017} = 1.058$$

This fact must be considered carefully by those who tend to reduce or eliminate the head space in containers and then are surprised to find that such containers explode or warp during sterilization.

Solutions (especially if filtered under gas pressure) contain considerable amounts of dissolved gases which leave the liquid phase as the temperature rises.

The overpressure of approximately 1.40 bar which occurs in the bottle naturally generates a force of approximately 1.4 kg per cm^2 of internal surface of the bottle. A rubber stopper with a diameter of 24 mm is subjected to an expulsion force of approximately 6.3 kg.

These conditions therefore prevent or advise against the use of a pure saturated steam autoclave to sterilize solutions contained in a wide variety of containers. For example

Large-Volume Parenterals (LVP) in glass containers
Small-Volume Parenterals (SVP) in glass vials with rubber stopper
LVP or SVP in plastic containers (flexible, semirigid or rigid plastic)
Prefilled syringes
Jars or similar containers with press-on or screw on closures
Blisters containing various materials, eg, disposable contact lenses.

Two counterpressure methods currently in use are

Superheated water spray method (sometimes named "water cascade process").
Air overstream method (sometimes named "steam + air method").

Superheated Water Spray Autoclaves—A typical functional diagram of this autoclave is shown in the Fig 5. Obviously, different solutions are also possible which, however, do not change the essence of the method.

The chamber generally has a circular crosssection (with quadrangular door(s) inscribed in the circumference) and has a single wall.

At the beginning of the program, after the goods have been loaded, the lower circular sector is filled with purified water. The air contained in the chamber is *not* removed.

The water, drawn by a sanitary-type pump, circulates in a heat exchanger (plate or other sanitary type) which is *indirectly* heated in countercurrent with industrial steam. The water returns then into the upper part of the chamber and is distributed to the load by a system of solid-cone spray nozzles. The uniform redistribution of the water on the lower layers of the load is ensured by appropriate perforated racks which support the load. Side spray bars sometimes are used, even if their actual usefulness is not demonstrated.

The heating of the circulation water, and therefore of the load, is gradual but quite fast; eg, the temperature of 121° is reached in approximately 20 to 30 minutes *inside* 500-mL containers, mainly dependent on the solution and the material and shape of the containers.

The sterilization phase lasts 15 to 20 minutes, and temperature uniformity (in time and space) is excellent: it is well

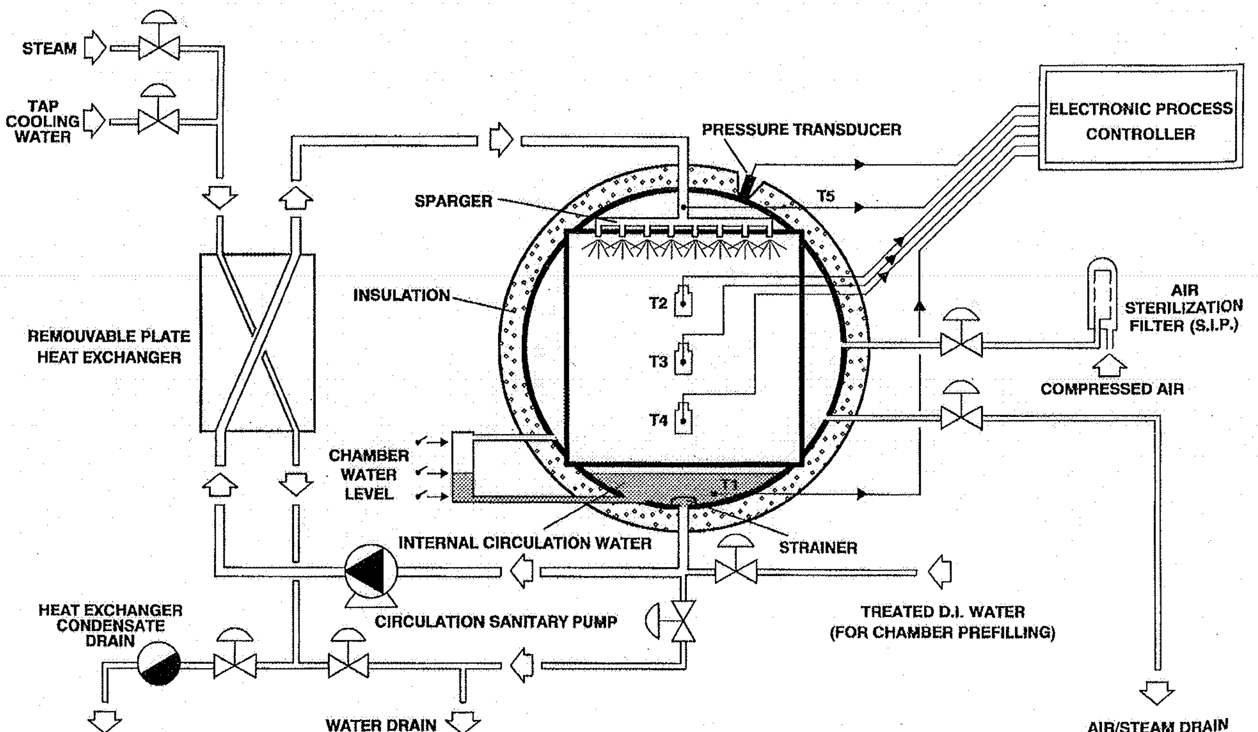


Fig 5. Superheated water-spray autoclave: simplified piping and instrumentation diagram (courtesy, Fedegari Autoclavi).

within the quite narrow limits required by FDA for LVP sterilization, ie, ±0.5°. This allows very small F_0 dispersions and therefore minimum sterilization times.

The cooling phase is performed while the circulation water, now sterile, continues to circulate. However, cold tap water now flows in the plates of the exchanger, where steam was flowing earlier. In less than 15 minutes, the temperature *inside* the 500-mL containers drops to approximately 70°, which is also the ideal temperature for obtaining a rapid and spontaneous drying of the load removed from the autoclave.

During all the phases of the process, an appropriate sterile air counterpressure is maintained inside the chamber to counterbalance the overpressure in the bottles.

There are various methods for controlling this counterpressure in each phase. With computerized management, it is even possible to generate a total pressure (steam + air) inside the chamber which is correlated, in each phase, to the average of the internal temperatures of two or more *witness* containers.

The load suffers no thermal or pressure shock and the differential pressure between containers and chamber can be eliminated or maintained in a direction convenient, in each phase, for the particular type of load. Even highly deformable products (semirigid plastic containers or plastic-aluminum blisters) or products which are particularly sensitive to differential pressures (eg, prefilled syringes) can be treated (from 60 to 127°) without problems.

The autoclaves are obviously highly specialized machines, and as such they have some limitations in application, such as

It is illogical to attempt to dry the load *inside* the autoclave by putting the chamber in vacuum or by circulating warm air.

In the case of materials with concavities directed upward, these concavities will be filled with water at the end of the program: the most obvious solution is to load these materials upside down.

When PVC bags are sterilized, the phenomenon of *blushing*, ie, the whitening of the PVC due to water absorption, usually occurs.

The intensity of this phenomenon and the time required for its disappearance depend on the type of PVC and of plasticizer employed. Blushing does not occur with rigid or semirigid plastic or with polylaminate plastics; it also is reduced considerably with PVC containing special plasticizers.

Air Over Steam Autoclaves—A typical functional diagram of this type of autoclave is shown in Fig 6. Alternatives are also possible in this case. The most important one is the use of horizontal faus placed on a side of the chamber. As in the previous case, the chamber has a circular cross section (with quadrangular door(s) inscribed in the circumference) and has a single wall.

There are two reasons for choosing a circular cross-section for autoclaves operating in counterpressure.

These autoclaves operate at significantly higher pressures than conventional pure saturated steam autoclaves, and generally are not put in vacuum. It is well known that a cylindrical structure withstands internal pressure much better than a quadrangular one.

The circular sectors of the chamber which are not occupied by the load are used to place elements required for the operation of these autoclaves.

The air is *not* removed initially from the chamber. The steam enters directly into the chamber through a sparger which is located in the chamber's lower portion.

The partial air pressure of the mixture can be adjusted during the entire process, similarly to what occurs for the previously described superheated water spray autoclaves.

The fan(s) placed against the ceiling of the chamber and the flow deflectors have the purpose of homogenizing the steam + air mixture which forms inside the chamber.

The task of these fans is very important and demanding. In fact, for equal pressure and temperature conditions, the air is approximately 1.6 times denser than the steam (one only has to consider their respective molecular weights) and would tend to stratify on the bottom, producing intolerable temperature gradients.

The cooling phase consists of feeding air into the chamber (in order to condense and replace all the steam which is present) while maintaining the same sterilization pressure or possibly increasing it. Cold tap water then is fed into the

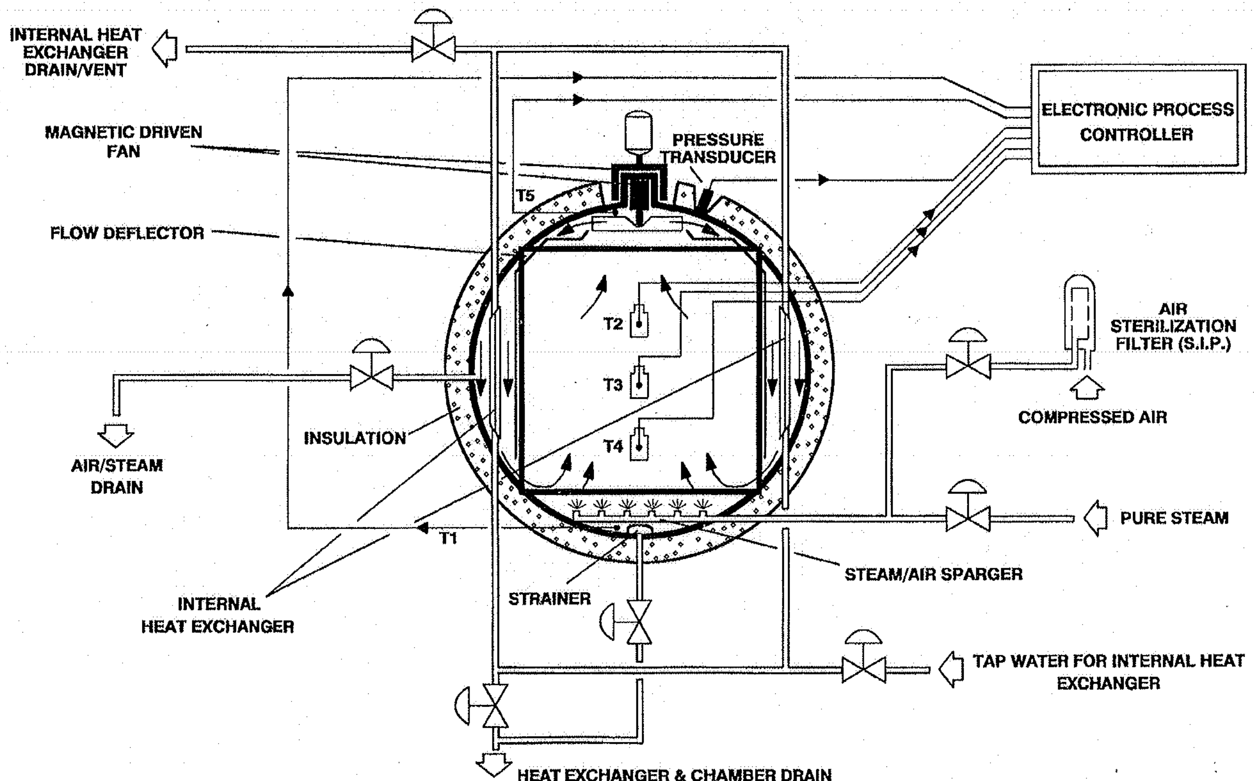


Fig 6. Air over steam autoclave: simplified piping and instrumentation diagram (courtesy, Fedegari Autoclavi).

heat exchangers, which are constituted by batteries of hollow plates located in the two circular sectors of the sides of the chamber (only one plate is shown in the diagram for the sake of simplicity). A tube heat exchanger can be used as an alternative.

The load is thus cooled while constantly maintaining a controlled pressure inside the chamber. However, this cooling comprises two solid-gas heat exchanges (plates → air; air → load) which, as is known, have a very poor efficiency. An attempt is made to improve this exchange by increasing the pressure of the air in the chamber (within the limits allowed by the product and the autoclave) so as to increase its density and therefore its heat-exchange capacity. The fans obviously continue to run during the cooling phase.

Despite these refinements, the cooling phase is definitely longer than the same phase in superheated water spray autoclaves.

A critical mechanical aspect of these autoclaves is the tightness of the fan shaft. This aspect can be solved completely by using magnetic-drive fans.

With steam + air mixture autoclaves, the blushing of PVC bags is less intense than with water spray autoclaves and generally affects essentially the regions where the bag rests on the supporting racks.

Table 1 compares the characteristics of the two kinds of counter pressure autoclaves.

Dry Heat Treatments

Sterilization and Depyrogenation—Dry heat treatments have two targets: microorganisms and their by-products. The aim of sterilization is to destroy the ability of microorganisms to survive and multiply. Depyrogenation seeks to destroy the chemical activity of the by-products: pyrogens or endotoxins (these terms do not mean exactly the same thing, but we will consider them to be synonymous for the sake of simplicity).

Both processes consist of an oxidation which is almost a combustion. However, the temperatures required to achieve depyrogenation are distinctly higher than those needed to obtain sterilization. We can summarize the situation as

If an effective dry heat depyrogenation is performed, sterilization generally is achieved *as well*.

Effective dry heat sterilization can be performed even *without* achieving depyrogenation.

If moist heat sterilization is performed, in normal operating conditions depyrogenation is *not* achieved.

The kinetics of dry heat treatments is not substantially different from that of moist heat sterilization. The values of the algorithms F_T and F_H (analogous to F_0) and those of the parameters D and z, however, are different not only from those of moist heat sterilization but also from each other. Furthermore, the two dry heat treatments are verified biologically with different biochallenges. Accordingly, the two dry heat treatments require different validation approaches.

The materials subjected to dry heat treatments naturally must be heat-stable: the most common are glass containers for parenterals. Elastomeric compounds generally are unable to tolerate these treatments.

The literature generally mentions the following operating conditions:

Sterilization:	160°—120 to 180 minutes 170°—90 to 120 minutes 180°—45 to 60 minutes
Depyrogenation:	230°—60 to 90 minutes 250°—30 to 60 minutes

However, the current trend is towards using treatments at higher temperatures than those listed.

The sections that follow describe the most common types of equipment used to perform the above-mentioned processes. The following general remarks should be made regarding this equipment:

If the load (bottles/vials/ampuls made of glass or other materials) is wet when it is introduced, a large part of the energy required by the process is used initially to evaporate the water that wets the load, and the process accordingly takes more time.

Table 1.

Critical comparison	Water spray autoclaves = WS	Air over steam autoclaves = AS
Temperature uniformity in time	Very good } easily in ±0.5°C limits requested by FDA for LVP	Very good } easily in ±0.5°C limits requested by FDA for LVP
Temperature uniformity in space	Very good	Very good
Total pressure uniformity in time	Very good	Very good
Counterpressure management flexibility	Excellent	Excellent
Consumption of high microbiological quality water	Yes, modest, for initial filling	No
Consumption of tap water for cooling	Yes, acceptable	Yes, approx. 3 times higher than WS
Consumption of compressed air	Yes, acceptable	Yes, acceptable
Consumption of industrial steam	Yes, acceptable	No
Consumption of ultraclean steam	No	Yes, acceptable
Condensate recovery	Possible and easy	Not possible
Cooling water recovery	Possible, recovered water is initially very hot	Possible, recovered water is initially very hot
Autoclave price	Acceptable	Approx. 1.1 times higher than WS
Total process duration	Short	Approx. 1.3 times higher than WS
Autoclave productivity/price	High	Approx. 70% of WS
Operating principle	Very simple and straightforward	More complicated than WS
Mechanical construction	Simple	More complicated than WS
Qualification/validation	Normal	Normal
Operating flexibility according to type of load	Suitable for any kind of container with the following remarks: · Upward concavities collect water · Product is unloaded wet · PVC bags can produce blushing phenomena	Suitable for any kind of container: · Upward concavities collect condensate only · Other kind of container can be unloaded lighly damp · Blushing phenomena of PVC bags are limited
Possibility of combination with pure saturated steam processes	Strongly discouraged: it is complex and expensive and complicates validation	Very frequent, but moderately expensive

The equipment uses large amounts of air which generally is recirculated partially and must be filtered in HEPA filters in order to have, in the critical regions of the equipment, Class 100 environment which is indispensable to obtain a load which is not only sterile and depyrogenated but also has extremely low particulate contamination. This is relatively easy to achieve in the sterilization phases (or regions) in which the *thermal situation* of the filters is stable. It is much less easy to achieve in the heating/cooling phases (or regions), because the changes in temperature entail expansions/contractions of the filters, with consequent release of particles.

Dry Heat Batch Sterilizers—The forced-convection batch sterilizer is a type of dry heat unit widely used in the industry. It uses the principle of convective heat transfer to heat the load. Figure 7 is a schematic diagram of a modern unit. It shows a two-door sterilizer in which the unloading door leads to the sterile area. The two doors are, of course, parallel to the plane of the drawing and are hinged vertically.

The pressure inside the chamber must be controlled continuously so that it is slightly higher than the pressure in the loading area (nonsterile) and slightly lower than the pressure in the unloading area (sterile).

The unit is made entirely of stainless steel; particular care must be placed in selecting the insulating materials and in the methods for applying them. It is in fact important also to avoid the forming of so-called *thermal bridges* that allow dissipation and therefore excessive external temperatures of the sterilizer and *cold spots* in the chamber.

The main features shown in the sketch are

1. Air-circulation fan
2. Water-cooled battery (for the cooling phase)
3. Circulation HEPA filters
4. Launch/recovery bulkheads
5. Trolley and load
6. Discharge duct
7. HEPA filter on the discharge duct to prevent back-flow contamination
8. Variable-speed fan for chamber pressurization (proportionally controlled)
9. Prefilter and HEPA filter on the chamber pressurization loop
10. Electric heater (proportionally controlled)
11. Four flexible Pt100 4-wire RTDs
12. Main control Pt100 4-wire RTD
13. Pressure transducer

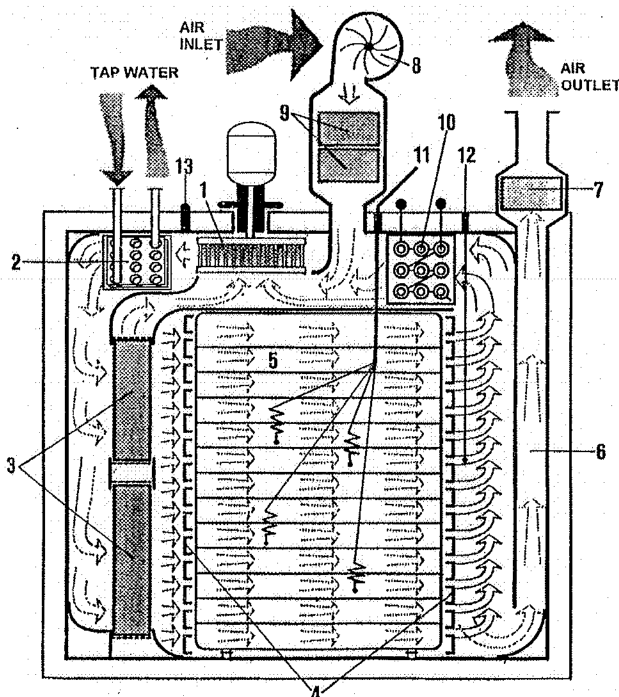


Fig 7. Dry-heat batch sterilizer: simplified diagram (courtesy, Fedegri Autoclavi).

Dry Heat Tunnels—The drying, sterilizing/depyrogenation, and cooling tunnel is the only continuous sterilizing apparatus widely used in the pharmaceutical industry (apart from filters). It basically consists of a horizontally rotating transport belt made of a stainless-steel mesh (some devices must be provided to confine the product on the transport belt without particulate generating friction), installed in a thermally insulated "tunnel" directly connecting an upstream cleaning machine to the downstream sterile area or to "isolated" devices.

Inside the tunnel, the product (most frequently glass vials) is dried, heat-treated either by radiant heat or, as more usual today, by hot air and finally cooled. In both cases the internal part of the tunnel must be pressurized dynamically by ventilation at an intermediate pressure level between the downstream system and the loading room. From a process point of view, higher temperature and shorter exposure time are used than in batch sterilizers. During the last 10 years the practice has changed from 20 minutes at 280° to 3 or 4 minutes at 300° or more. Since a minimum safety margin is required for the duration of exposure, and glass of most types becomes more difficult to handle above 320° and more fragile after such a treatment, it is likely that the trend toward higher temperature values has reached its practical limit.

In IR (infrared radiant heat) tunnels, heat is supplied by resistance-in-glass heaters located above and below the transport belt; prefiltered and HEPA-filtered air is fed into the cooling zone mainly for pressurizing and cooling. This air, countercurrent slowly flowing through the entire tunnel, has also an important drying and preheating effect of the load in the infeed zone. Figure 8 schematically represents an IR tunnel: even if this type of apparatus is no longer widely used, the basic concepts have not been modified in the LF (hot air laminar flow) tunnel, but airflow patterns are a little more complex.

LF tunnels do not radiate heat directly to the product, but heating is provided by circulation of hot filtered air forced onto the product. A circulation fan withdraws the air, leaving the product through heating bars below the transport belt and feeds it again to the inside of the tunnel through HEPA filters suitable for operating at high temperature. Airtightness of the coupling of HEPA filters with tunnel framework is of utmost importance from the point of view of particulate contamination. It must cope with the strong thermal expansion of different materials. Some makeup air is required in the heating zone, and the total number of installed fans may be as high as five, or six if an additional extraction below tunnel outfeed is required in case of high pressure in the sterile room.

Despite the complexity of its airflow, the LF tunnel has the main advantage of quicker heating and consequent shorter process time. This results in reduced size compared with the IR tunnel, since the belt speed cannot be reduced below a certain value. As the name itself declares, the air speed in the LF tunnel is kept around 0.5 m/sec (1.5 ft/sec), aiming to avoid particulate contamination.

The comparison between continuous tunnel and batch oven is favorable to the continuous tunnel from the point of view of handling the product. No batch work is needed after the unpacking of the components and loading of them into the cleaning machine until the final removal of the packaged product from the line after the filling and the following operations.

This can be very important in the case of large-scale production, but the batch oven provides a much easier isolation of the sterile area. In the case of continuous tunnel, there must be a steady flow of air through the open connection from the sterile area to the tunnel. The pressure difference between the two systems must be such that the sterile area always is kept at a higher pressure level than the tunnel. Too big a difference would result in an excessive escape of air to the tunnel, both reducing the pressure in the sterile area and disturbing the laminar air flow and the temperature profile inside the tunnel.

Experience has proved that these problems can be solved satisfactorily only if the design of the air-conditioning system

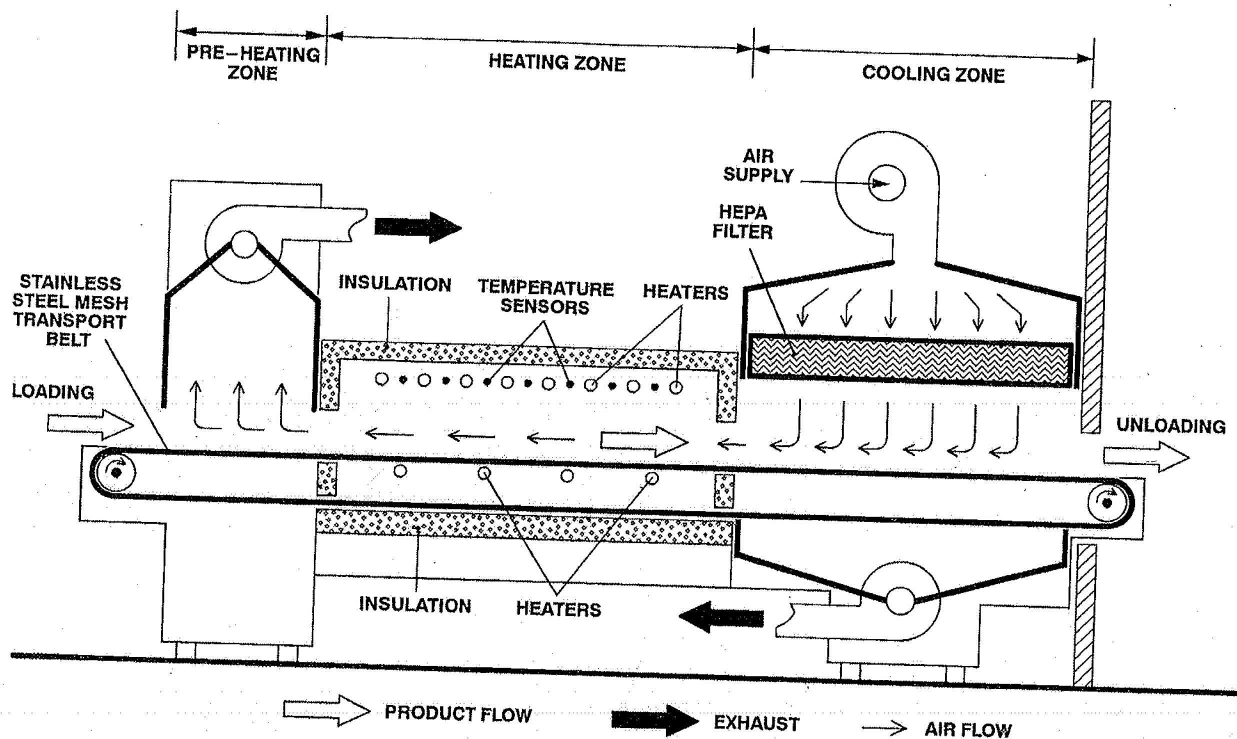


Fig 8. Dry-heat tunnel: simplified diagram (courtesy, Fedegari Autoclavi).

of the sterile area is developed from the very beginning keeping in mind the foreseen installation of a specified tunnel. Baffle systems also aid in maintaining pressure differentials between the aseptic-processing area and the sterilizing tunnel.

Chemical "Cold" Sterilization

Many products do not tolerate the sterilization conditions of moist-heat or dry-heat processes. In such cases it is possible to resort to cold or at least low-temperature sterilization methods performed with chemical means, ie, gases or vapors.

The continuously increasing use of plastic disposable products or components for medical treatments has been made possible by the development of reliable cold sterilization processes.

A variety of gases and vapors have shown germicidal properties: ethylene oxide, propylene oxide, formaldehyde, betapropiolactone, ozone, hydrogen peroxide, peracetic acid, etc. Ethylene oxide (EtO) is currently in widespread use for medical product sterilization. Vaporized hydrogen peroxide and hydrogen peroxide/steam mixtures are being used to sterilize a variety of materials and work surfaces.

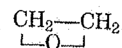
Ethylene Oxide—The sterilizing action of EtO is based on an alkylation reaction: it is, accordingly, a truly chemical action rather than a physical one. This chemical reaction must be activated by the presence of water vapor (approximately 60% of RH or relative humidity) and is increased by temperature and EtO concentration.

Process temperature is limited by the characteristics of the product. Generally, it is between 40 and 60°, but it must be remembered that the reaction rate increases by approximately 2.5 times for each 10° increase in temperature. Normally used EtO concentrations range between 400 and 1200 mg/L. It has in fact been demonstrated that beyond 1200 mg/L the consequent increase in the reaction rate is no longer economically convenient.

The EtO must make *direct* contact with the microorganism for the microbe to be inactivated. Any packagings that contain the object to be sterilized must therefore be permeable to air, EtO and any dilution gases (as mentioned later). Generally, it is not possible to use EtO to sterilize liquids, solutions, emulsions, etc. Powders, too, are difficult to treat unless microbial contamination is only on the outside of the granules.

Fortunately, EtO, air and dilution gases easily penetrate most of the plastic and paper barriers used for the packaging of medical products. However, the good penetrating properties of EtO are also a disadvantage, since large amounts of it are absorbed by plastic or rubber materials. Products sterilized on an industrial scale using EtO normally require about 14 days of quarantine to spontaneously eliminate absorbed EtO residuals. This time can be reduced by using forced desorption methods. Sterilized goods must be monitored for toxic EtO residual, ethylene glycol and ethylene chlorhydrin-breakdown products of EtO.

EtO in standard room conditions is a vapor (indeed, its boiling point is about 11° at atmospheric pressure). It is colorless, heavier than air, and has an ether-like odor. Its formula is



$$CH_2\text{—}CH_2 \text{ (bridged by O)}$$

The presence of the oxygen bridge, which can be opened easily, explains its reactivity and its sterilizing action, as well as its tendency to polymerize.

Unfortunately, EtO has several drawbacks: it is toxic, carcinogenic, teratogenic, inflammable and explosive when mixed with more than 3% air by volume. These characteristics make it highly controversial to use EtO, and many countries have issued regulations or requirements for its use as a sterilizing agent.

EtO often is used in a mixture with dilution gases, with weight ratios of 85 to 90% of diluent. The diluent gas most often used is CO_2; use of freon is shrinking, due to the well-known international restrictions to its use; N_2 is beginning to be used quite often.

These mixtures are considered nonflammable and nonexplosive, and many countries consider them mandatory for use in industrial autoclaves. These countries allow the use of pure EtO in small individual single-use cylinders only for small autoclaves (100 to 200 L). If a 10 to 12% mixture of EtO in CO_2 is used to obtain an acceptable EtO concentration (at least 500 mg/L), it is necessary to work at 3 to 4 absolute bar. Accordingly, one must use an autoclave that can withstand relatively high pressures and is therefore expensive, and the duration of the sterilization process is rather long because of EtO concentration is rather low.

Other countries accept the use (including industrial use) of pure EtO or of inflammable/explosive mixtures with a low percentage of dilution gas (the presence of the dilution gas generally is ascribed to a reduction in the tendency of pure EtO to polymerize). In such circumstances one can operate at less than atmospheric pressures and still reach high EtO concentrations that shorten the sterilization time. It is therefore not necessary to use true autoclaves, but merely sterilizers capable of tolerating the very hard vacuum required for the initial elimination of the air from the chamber and from the load and for the final extraction of the EtO. Obviously, in these circumstances the use of plants constructed with explosion-proof criteria cannot be avoided.

The P/T/t diagrams of EtO sterilization are therefore different, depending on whether one or the other of the above described principles is used. A typical diagram of an overpressure sterilization with a mixture using 10% EtO and 90% CO_2 is shown in Fig 9. The steps are

Load and/or chamber heating
Vacuum
Vacuum hold for leak test
Humidification by steam injection
Penetration of humidity in the load
Loading of EtO mixture
Sterilization
EtO mixture evacuation
Air/vacuum pulses
Vacuum hold
Vacuum breaking

A typical diagram of a subatmospheric sterilization with a mixture using 85% EtO and 15% CO_2 is shown equally in Fig 9. One can see clearly that the phases are substantially the same as in Fig 8; what changes is the sterilization pressure, the EtO concentration and therefore the duration of the sterilization phase.

In performing industrial sterilizations, which accordingly involve large loads, the load is heated and humidified before placing it in the sterilizer, in adequately conditioned rooms. The heating/humidification phases described above in Figs 8 and 9 diagrams therefore are reduced drastically.

The layout of an industrial EtO sterilization plant is shown in Fig 10. Some remarks should be made regarding some of the items of this layout. This unit contains

The EtO or EtO-mixture cylinders.
The automatic devices that connect/disconnect the various cylinders to and from the sterilizer; disconnection of a cylinder (especially for mixture cylinders) often is controlled by its weight reduction, which must accordingly be checked individually.
The heat exchanger that must provide the vaporization calories to the liquid EtO mixture.
The pressure reduction unit that bring the liquid EtO mixture to the vapor state.
Any cylinders of N_2, which is used in the most advanced plants to "wash," after each process, the pipes that have carried EtO.

The EtO that is produced in the desorption chamber is at a very low concentration and it is generally too expensive to eliminate it with a catalytic burner. It is preferred to absorb it on activated-charcoal columns through which the air of the desorption chamber is recirculated.

Obviously, the EtO discharged by the sterilizer (and possibly the EtO arriving from the desorption chamber) must not be discharged into the atmosphere. Catalytic burners generally are used today: they convert the EtO into $CO_2 + H_2O$. These burners must be highly efficient, and their efficiency must be checked systematically, since the laws enforced in the various countries are generally very strict as to the limits of residual EtO. The asterisks (*) in the figure indicate points where continuous monitoring of EtO concentration must be provided.

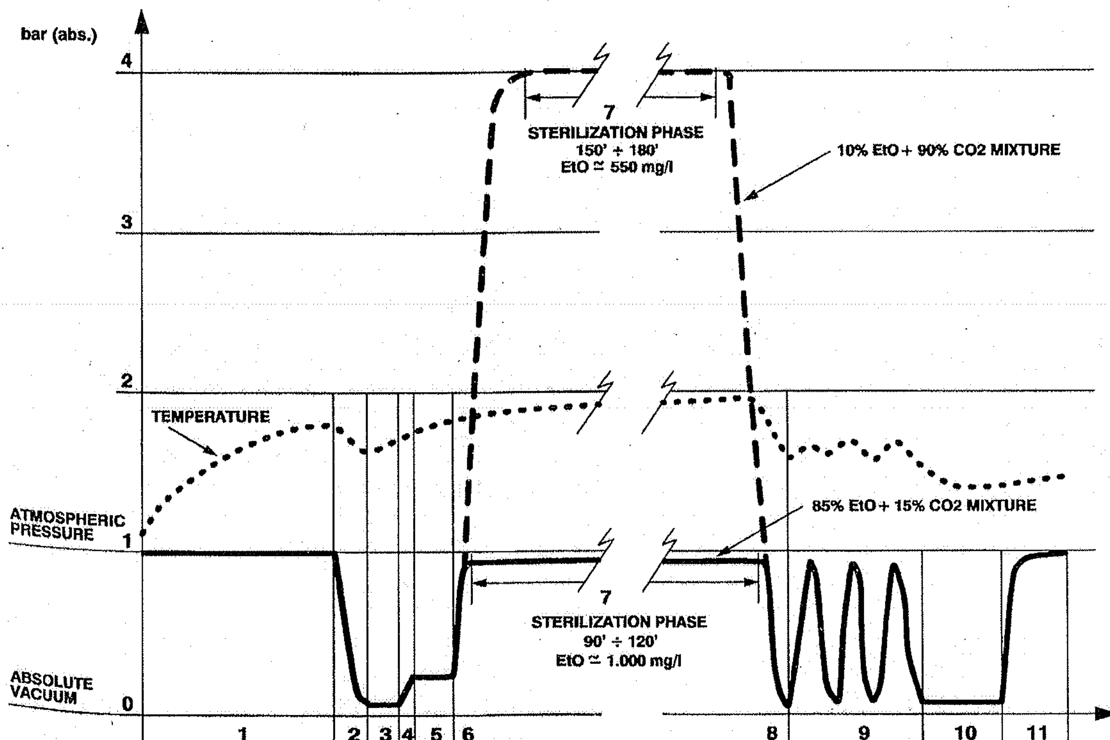


Fig 9. EtO sterilization pressure-time diagram: overpressure and subatmospheric pressure (courtesy, Fedegari Autoclavi).

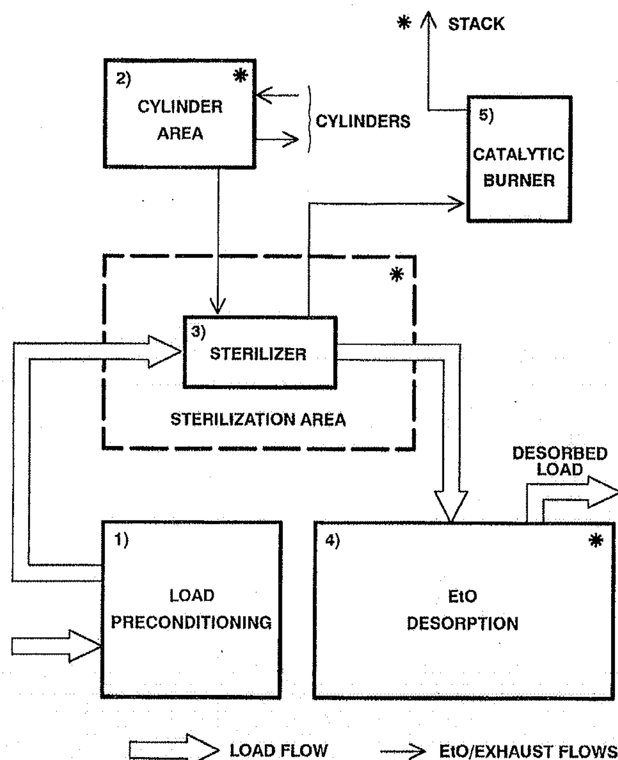


Fig 10. Flow diagram of an EtO sterilization industrial plant (courtesy, Fedegari Autoclavi).

EtO sterilizers generally are made of stainless steel, although there also are machines made of carbon steel coated with epoxy paints. The chamber generally is jacketed in order to circulate thermostat-controlled warm water to maintain the sterilization temperature. Use of water vapor for the same purpose is fading due to difficulties in using this method when the temperature must be kept below 100°.

Process sensors are more numerous than in heat sterilizers, since there are essentially four sterilization parameters:

EtO concentration
Temperature
Humidity or RH
Time

The EtO concentration generally is monitored by the pressure rise that occurs in the chamber when the EtO mixture is introduced; a pressure transducer is therefore used as a sensor.

Many Guidelines require, in addition to the pressure rise, a second monitoring method that can be chosen among

1. Weight difference of gas cylinder
2. Volume of gas delivered
3. Sampling from the sterilizer chamber and analysis

When EtO mixtures are used, Methods 1 and 2, like the pressure rise method, assume confidence in the concentration of EtO that is present in the mixture and that reaches the sterilizer.

Method 3 is certainly more reliable, but it also is more difficult to apply. Infrared spectrometry or gas chromatography methods generally are used for analysis; these methods can be continuous and allow the simultaneous determination of the relative humidity (RH).

Temperature generally is monitored by temperature sensors which are located in the chamber and may be placed inside the load as well. The relative humidity (RH) generally is monitored on the basis of the temperature and of the pressure rise of the steam injection of the humidification phase. This method obviously is not very reliable, and many guidelines recommend also to use a sensor that can determine the RH. Unfortunately, sensors of this kind are generally "poisoned" by the EtO and become unreliable after a few cycles. The solution often used is to keep the sensor inside the chamber during the humidification phases, removing it before introducing the EtO in the chamber.

Finally, it is evident that if the load is preconditioned, the preconditioning temperature/humidity/time also must be monitored.

Hydrogen Peroxide—Hydrogen Peroxide (HP), chemically H_2O_2, is normally a liquid at room temperature. However, it can be vaporized and the resultant gas is an effective sterilant for certain packaged materials and for equipment and enclosures used in processing sterile materials. The most frequent and successful use of HP as a sterilant is for isolators (also known as barriers, locally controlled environments, etc). These units are very sophisticated versions of their ancestors ("glove boxes"), which were used to isolate processes in the past.

Isolators now are used widely for sterility testing, transporting sterilized goods from moist and dry heat units to sterile areas or processing isolators and processing of supplies. HP also is being used to sterilize more sophisticated processing equipment, such as freeze dryers and filling lines, and even may be used to sterilized small clean rooms. High humidity can inhibit the effectiveness of vaporized HP and must therefore be controlled during the exposure of the gas. Figure 11 represents a typical vaporized HP cycle.

While HP is broken down readily to water and oxygen, the effluent gas can represent a safety hazard at higher levels. Just as with EtO, catalytic converters are used to assure that all materials are rendered safe before they are released to the atmosphere. Figure 12 represents a typical installation using vaporized HP to sterilize a freeze dryer and condenser system. VHP DV1000 is a model manufactured by the Am Sterilizer/Finn Aqua Co, who hold many of the patents on the use of this technology.

Systems for larger applications may require fans to aid in uniformly distributing the vaporized HP. In addition, auxiliary air systems may be added to reduce the time required to dehumidify at the beginning of the cycle and to aerate the load at the end of the cycle. Figure 13 shows a transfer isolator connected to a sterilizer and a vaporized HP generator. This particular unit also has a protective half-suit to allow full access to the large internal area. These units allow the unloading of the sterilizer directly into a sterilized isolator. The isolator excludes direct human intervention, which greatly reduces the potential for microbial contamination.

A typical freeze-dryer sterilization involves several vacuum "pulses" during which the temperature is brought to 40 to 60° and the humidity is reduced (dry phase). A vacuum hold cycle is run to check for leaks and the temperature is reduced to about 25° for the sterilization cycle. The sterilant is introduced and is monitored and controlled by weight using an electronic balance. Filtered air is pulsed with sterilant to push the vapors into any deadlegs and to compress the vapors, thus increasing the concentration. Finally, the vacuum is pulse again to aerate the chamber, and the residual vapor is verified to be below acceptable levels before proceeding to the processing cycle.

Hydrogen Peroxide Plus Steam—For certain applications, one can combine moist heat and hydrogen peroxide methods. The combination can produce some effects which may be more desirable than either of the techniques run separately. Cycles can be as effective in shorter times and may improve the removal of residual peroxide. The system must be able to withstand exposure to steam at atmospheric pressure. The air-handling equipment can be moved outside the processing area, which simplifies the system and minimizes any mechanically generated particles, since the air, steam and peroxide are introduced through the same type of HEPA filters used for laminar-flow hoods.

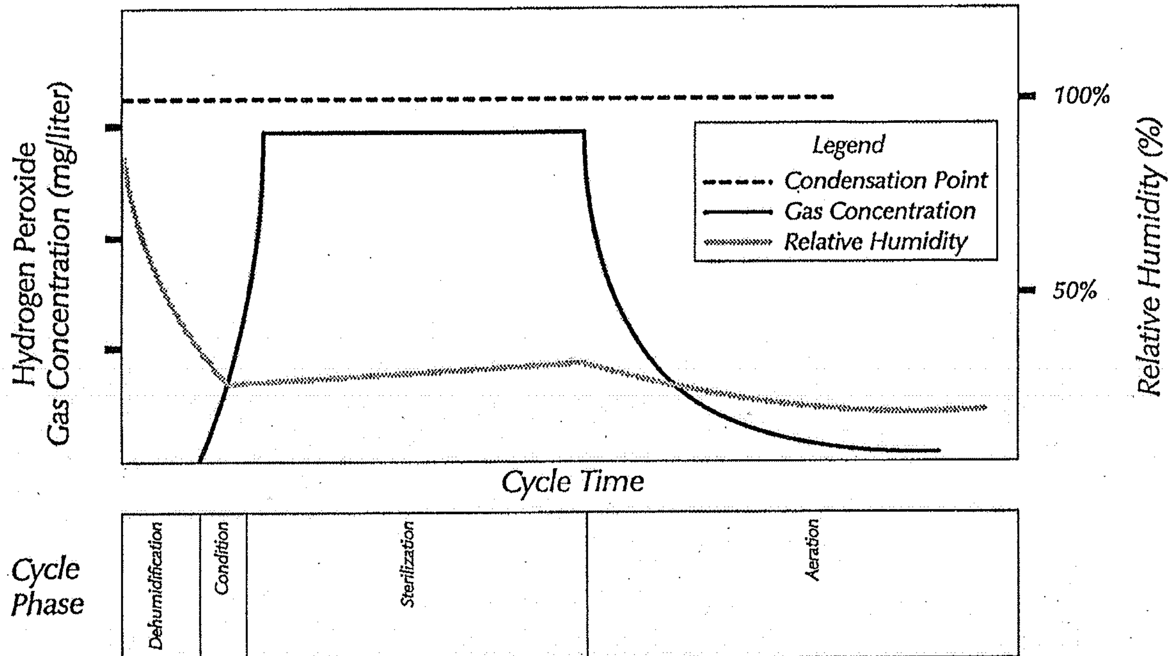


Fig 11. A typical vaporized HP cycle (courtesy, Am Sterilizer/Finn Aqua).

The process is basically as follows:

The process area is raised to about 80° by introducing dry heated air through the HEPA filters. The steam is introduced and surfaces are raised to about 100°. During the steam cycle, hydrogen peroxide is introduced and is carried with the steam. When the cycle has been completed, the steam and peroxide are stopped and the dry heated air is started again. This aids in removal of residual condensate and helps break down the peroxide to water and oxygen. After sufficient heat has been introduced to dry and remove residuals, cool air is introduced to bring the unit to the desired operating temperature.

Since the hydrogen peroxide is mixed intimately with the steam, temperature can be used to monitor the progression of the cycle. However, the heated portions of the cycle must be validated using biological indicators and residual peroxide measurements, to assure their effectiveness in sterilizing and removing residuals to a safe level. Figure 14 diagrams a cycle using steam and hydrogen peroxide to sterilize as a filler in an isolator.

Figure 15 is included to show the synergistic effects of steam and hydrogen peroxide in some sterilization cycles. The challenge organism was *Bacillus stearothermophilis*, which typically is used to validate steam cycles. It should be noted that the kill rate was not only considerably faster, but was accomplished using atmospheric steam. This means that instead of 121° the equipment was only subjected to 100° and was exposed for 15 minutes less to achieve the same reduction in microorganism count.

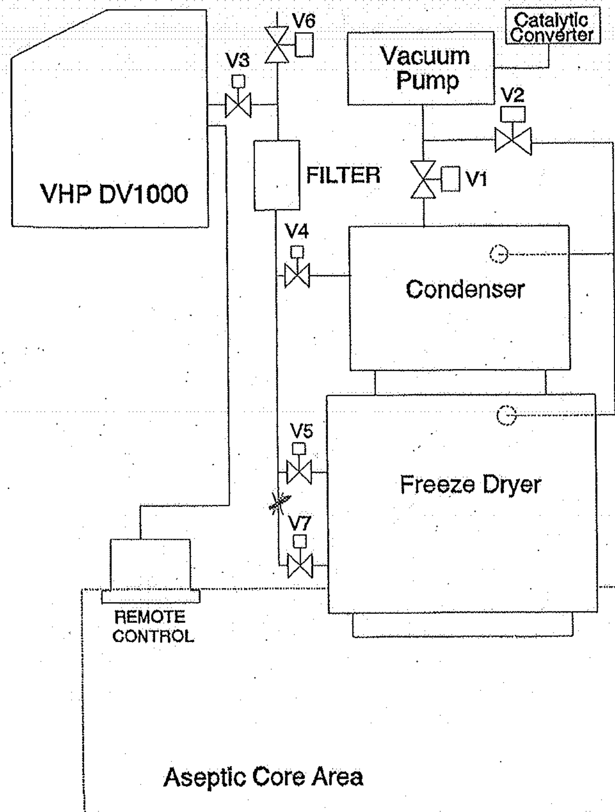


Fig 12. A typical installation using vaporized HP to sterilize a freeze dryer and condenser (Courtesy, Am Sterilizer/Finn Aqua).

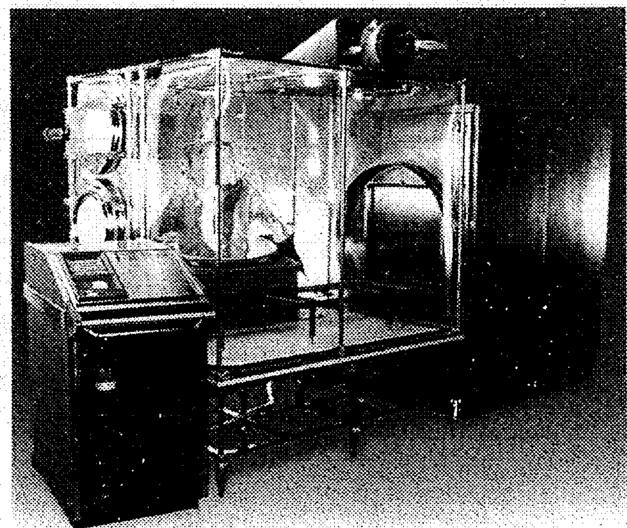


Fig 13. A transfer isolator connected to a sterilizer and a vaporized HP generator (Courtesy, Am Sterilizer/Finn Aqua).

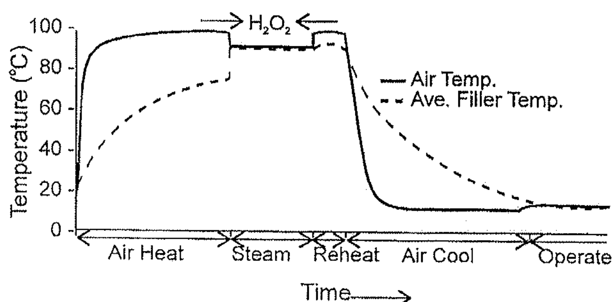


Fig 14. A steam/hydrogen peroxide cycle (courtesy TL Systems and Despatch Industries).

Other Gases—Formaldehyde (HCHO) sometimes is used for sterilizing certain medical products. It is not in widespread use in the US but as a gas or in combination with low-pressure steam, it is used in some European hospitals instead of ethylene oxide. Formaldehyde, a toxic chemical and a human carcinogen, is an alkylating agent and destroys microorganisms by alkylation of susceptible cell components.

Chlorine dioxide (ClO_2) is an effective antimicrobial agent in both liquid and gaseous states. Its use as a gaseous sterilant has been considered impractical because the gas could not be shipped or stored. There have been, however, some recent innovations allowing for *in situ* generation of the gas, which would make possible its future use in a sterilization chamber.[11]

Filtration

Filtration is the removal of particulate matter from a fluid stream. Sterilizing filtration is a process which removes, but does not destroy, microorganisms. Filtration, one of the oldest methods of sterilization, is the method of choice for solutions that are unstable to other types of sterilizing processes.

Pasteur, Chamberland, Seitz and Berkfeld filters have been used in the past to sterilize pharmaceutical products. These types of filters were composed of various materials such as sintered glass, porcelain or fibrous materials (ie, asbestos or cellulose). The filtration mechanism of these depth filters is random adsorption or entrapment in the filter matrix. The disadvantages of these filters are low flow rates, difficulty in cleaning and media migration into the filtrate. Fiber-releasing and asbestos filters now are prohibited by the FDA for the filtration of parenteral products.[12,13]

Over the past 30 years, membrane filters have become the method of choice for the sterilization of heat-labile sterile products. Membrane filters are thin, strong and homogenous polymeric structures. Microorganisms, present in fluids, are removed by a process of physical sieving and are retained on or near the membrane surface. Membrane filters of 0.22-μm pore size are employed commonly as sterilizing filters. However, 0.45-μm pore size filters are used to remove bacteria from antibiotics or steroids in organic vehicles prior to an aseptic crystallization process.

When solutions are sterilized by filtration, the filters must be validated to assure that all microorganisms will be removed under known conditions. Filter manufacturers normally validate sterilizing membrane filters using a protocol similar to the one developed by the Health Industry Manufacturers Association (HIMA).[14] In this procedure, *Pseudomonas diminuta* (ATTC 19146) is cultivated in saline lactose broth. Leahy and Sullivan[15] have shown that when *Pseudomonas diminuta* is cultivated in this medium the cells are discrete and small (approximately 0.3 μm in diameter)—a range recommended for sterilizing filtration with 0.22-μm filters. Each cm^2 of the filter to be validated is challenged with 10^7 microorganisms at a differential pressure of 30 psig. The entire filtrate is collected and tested for viable microorganisms. The retention efficiency (log reduction value) of the membrane filter may be calculated using the procedure described in the HIMA protocol. Dawson *et al*[16] have demonstrated that the probability of a nonsterile filtration with a properly validated membrane filter is approximately 10^{-6}. Another aspect in filter validation is adsorption of the product by the filter and extractables from the filter and housing.

Once the performance of the membrane filter has been validated, a nondestructive integrity test that has been correlated to the bacterial challenge test (the bubble point or diffusion test) can be used routinely prior to and after a sterilizing filtration to assure that the membrane filter is integral.[17,18] Unique to membrane filtration is the condition that beyond a certain challenge level of microorganisms, the filter will clog. For a typical sterilizing filter this level is 10^9 organisms per cm^2. Initially, membrane filters were available only in disc configuration. Advances in membrane technology have provided filters in stacked-disc, pleated-cartridge and hollow fiber configurations. These advances have provided larger surface areas and higher flow-rate capabilities. Figure 16 is an example of these larger surface area filters.

Membrane filters are manufactured from a variety of polymers; cellulosic esters (MCE), polyvinylidiene fluoride (PVF), polytetrafluoroethylene (PTFE), etc. The type of fluid to be

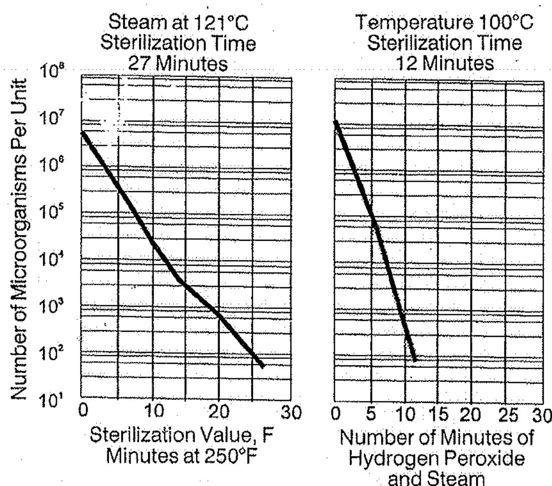


Fig 15. Comparison of steam under pressure with hydrogen peroxide/atmospheric steam mixture (courtesy, TL Systems and Despatch Industries).

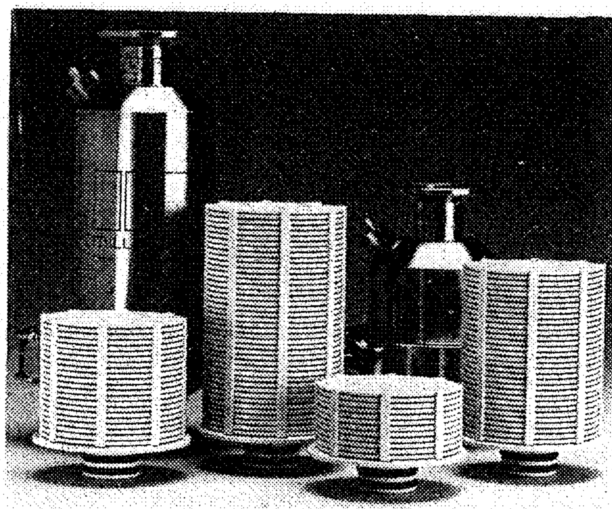


Fig 16. Stacked-disk membrane filters. This new technology allows filter manufacturers to supply filters with large surface area in relatively small packages (courtesy, Millipore). vaporized HP generator (Courtesy, Am Sterilizer/Finn Aqua).

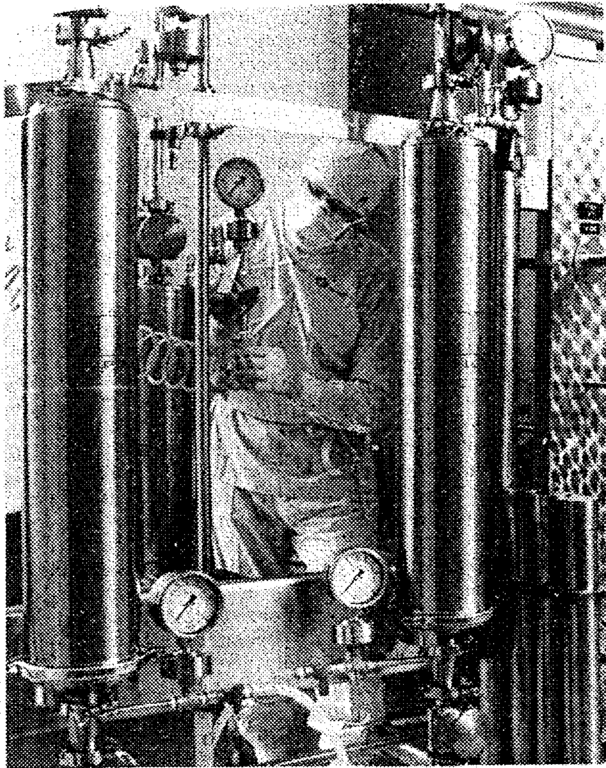


Fig 17. An example of a process filtration system in a pharmaceutical plant (courtesy, Millipore).

sterilized will dictate the polymer to be used. The listing below is intended to serve only as a guide for the selection of membrane filters for a particular application. The filter manufacturer should be consulted before making a final choice.

Fluid	*Polymer*
Aqueous	PVF, MCE
Oil	PVF, MCE
Organic solvents	PVF, PTFE
Aqueous, extreme pH	PVF
Gases	PVF, PTFE

Figure 17 is an example of a sterilizing filtration system commonly used in the pharmaceutical industry.

Positive pressure commonly is used in sterilizing filtrations. It has the following advantages over vacuum; it provides higher flow rates, integrity testing is easier and it avoids a negative pressure on the downstream (sterile) side of the filtrate, thus precluding contamination. Membrane filters are sterilized readily by autoclaving, *in-situ* steaming or by using ethylene oxide.

In addition to their use in the pharmaceutical industry, membrane filters are used in many applications in the hospital pharmacy. The membrane filters commonly used in these applications are small disposable units. Examples of these are shown in Figs 18 and 19. Typical applications for membrane filters in hospital pharmacies include sterilization of intravenous (IV) admixtures and hyperalimentation solutions, sterilization of extemporaneously compounded preparations, sterility testing of admixtures as well as in direct patient care (see Chapter 88).

Radiation Sterilization

The retail or hospital pharmacist probably has little opportunity to use radiation sterilization. However, they should be aware that many of the products sold in stores and used daily in hospitals are sterilized by this technology. Products such as contact lens solutions, bandages, baby bottle nipples and teething rings (the kind containing water/gel) are a few examples of the everyday type of product encountered in a Pharmacy. Several drugs, including some anticancer drugs also are sterilized *terminally* using gamma radiation.

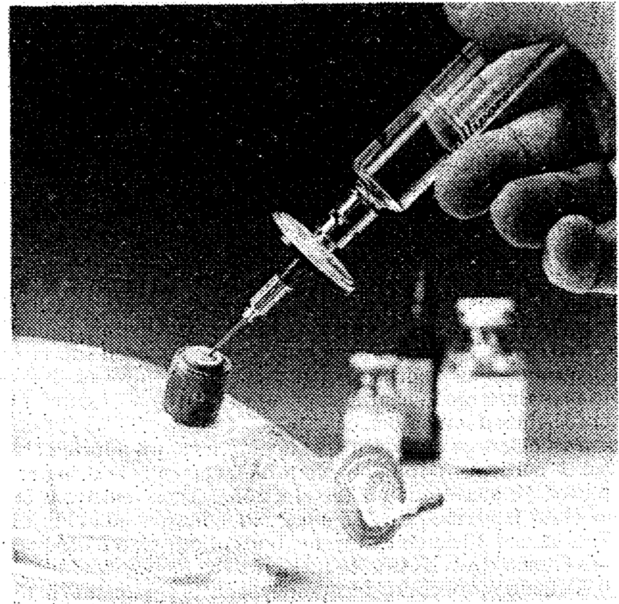


Fig 18. IV additive filtration using a small disposable membrane filter (courtesy, Millipore).

Fig 19. IV additive filtration and sterility testing. Both procedures employ membrane filtration (courtesy, Millipore).

The hospital pharmacist is likely to encounter the use of gamma or X-ray treatment of blood to eliminate white blood cells in host versus graft reactions following transplant surgery. The serum used for tissue cultures is frequently sterilized with gamma radiation to eliminate viruses, viruse-like particles and mycoplasmas.

The pharmaceutical industry historically has relied on steam, dry heat, ethylene oxide gas, filtration and chemical processes to meet sanitization or microbial load reduction requirements. Sterilization by radiation may employ either electromagnetic radiation or particle radiation.

Electromagnetic radiation, comprised of photons of energy, includes ultraviolet, gamma, X- and cosmic radiation. Gamma radiation, emitted from radioactive materials, such as $Cobalt^{60}$ or $Cesium^{137}$, is the most frequently used source of electromagnetic radiation. Of these two, only $Cobalt^{60}$ is used in the large industrial irradiator (Fig 20). $Cesium^{137}$ is used in blood irradiators.

Particulate or corpuscular radiation includes a formidable list of particles. The only one which currently is being employed for sterilization is the electron. These electrons are machine generated using the technique illustrated in Fig 21; Figs 22 and 23 illustrate two methods of presenting products to a commercial electron-beam sterilizer.

Radiation-processing technology, and its application in the manufacture of pharmaceuticals, is being investigated more actively now than at any other time. This renewed interest is in part due to the development of aseptic and barrier technology, as well as an overall improvement in the environment in which pharmaceuticals are manufactured.

In the past the use of a radiation dose of 25 kGy was required to ensure that all spores had been destroyed, and that a Sterility Assurance Level (SAL) of 10^{-6} was achieved. This level of radiation destroyed many pharmaceuticals. With the advent of clean rooms, and aseptic and barrier technologies, the microbial environment has been altered drastically. No longer are spores or even the *number* of organisms as daunting. It is more appropriate now to know the species and to tailor the radiation dose to meet the *species* bioburden. In this way many more drugs and other products are capable of being sterilized terminally. This provides an SAL of 10^{-6} or better, depending upon the organism.

The increased use of radiation processing to sterilize medical devices has led to the development of more efficient and economical irradiation equipment and processes. It also has generated new scientific data. The positive experience of the medical-device industry should be a "sign post" for the pharmaceuticals industry.

Several pharmaceutical raw materials and finished products are being sanitized/sterilized successfully with gamma radiation. While it is possible to use electron beam radiation, we are presently unaware of any pharmaceuticals being treated using this technology. This should not preclude others from investigating its potential. The unique penetrating ability of gamma radiation provides the edge for this technology in this application.

How Radiation Kills Microorganisms—The principles of sterilization by irradiation have been known since the early 1940s. Basically, charged particles or electromagnetic radiation interact with matter to cause both ionization and excitation. Ionization results in the formation of ion pairs, comprised of ejected orbital electrons (negatively charged) and their counterparts (positively charged). Charged particles such as electrons interact directly with matter causing ionization, whereas electromagnetic radiation causes ionization through various mechanisms that result in the ejection of

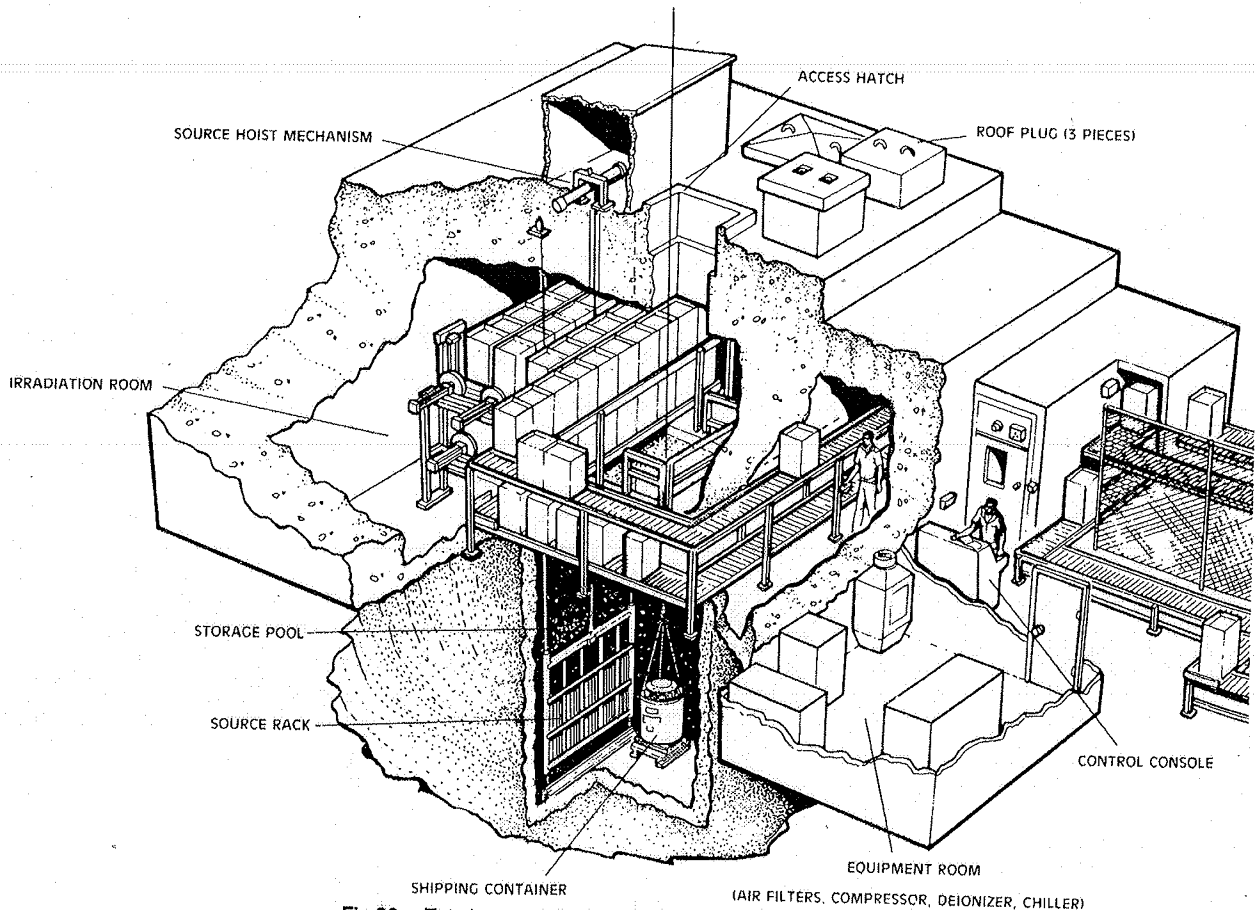


Fig 20. Tote box irradiator: automatic (courtesy, Nordion Intl).

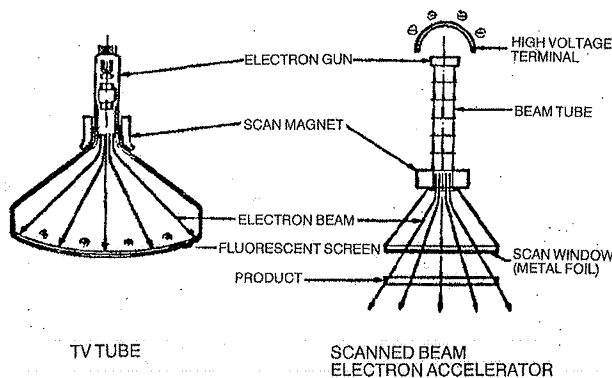


Fig 21. To produce an electron (courtesy, RDI).

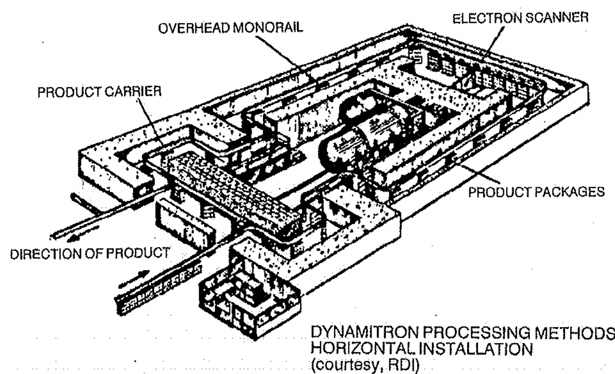


Fig 23. Dynamitron processing methods: horizontal installation (courtesy, RDI).

an orbital electron with a specific amount of energy transferred from the incident gamma ray. These ejected electrons then behave similarly to machine-generated electrons in ionization reactions. Thus, both particle and electromagnetic radiation are considered as ionizing radiation and differ from ultraviolet radiation in this respect.

Ionizing radiation kills or inactivates microorganisms through the interaction of the ion pairs or excitations altering the molecular structure or spatial configuration of *biologically active* macromolecules. In particular, those involved in cell replication are most critical. It can do this in two ways. The first is to deposit energy directly in a bond of the macromolecule. This can cause a rearrangement of its structure, altering or destroying its normal function. The second is to generate free radicals, primarily from the water contained within the cytoplasm. The free radicals thus generated react with the macromolecules to subvert their normal function. In either case the result is the loss of reproductive capability of the microorganism.

The number of organisms inactivated by a given radiation dose is a statistical phenomenon. It depends upon the sensitivity of the biologically active macromolecule(s) to alteration (denaturation), the number of alterations elicited within the cell and the ability of the cell to repair these alterations. Different organisms have different capabilities to withstand or repair such alterations. This sensitivity is referred to as the D_{10} value. The size of the organism, its state of hydration and the presence or absence of radical scavengers all affect the outcome of exposure to ionizing radiation.

The ability of gamma radiation to inactivate microorganisms has been well documented. New documentation relating to viruses, or new strains/reclassifications of microorganisms, is being added continually. The major benefit of using radiation sterilization as the terminal step in the manufacturing process as opposed to autoclaving, or dry-heat methods, is the lack of, or reduction in, product degradation with this technology.

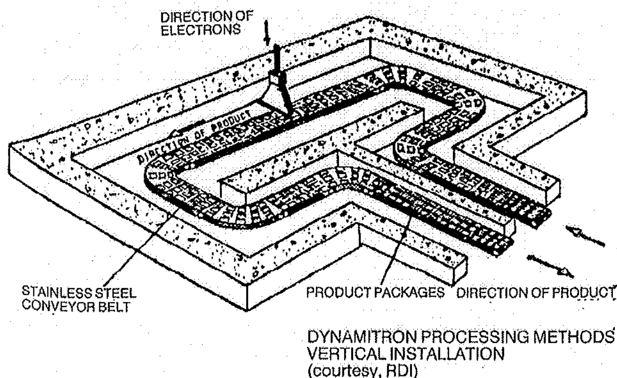


Fig 22. Dynamitron processing methods: vertical installation (courtesy, RDI).

The process has been in use in the medical device industry for over 20 years. Ample evidence as to its efficacy exists in the literature. Materials and processes have been developed to reduce the impact of radiation on the product. It is the intent of this update to present some of the process developments which will facilitate the use of this technology for the terminal sterilization of pharmaceutical products. It also will assist those wishing to improve the microbial quality of raw materials entering the manufacturing process. Clean materials reduce the bioburden impact on a clean room facility.

Sterilization by ionizing radiation requires consideration of the dose (or the amount of radiation that is absorbed by the material), the energy level available (which along with the bulk density of the material will determine the thickness of penetration) and the power output available (which determines the rate at which the dose can be applied).

The unit of absorbed dose is the Gray (Gy), where 1 Gy = 1 J/kg, independent of the nature of the irradiated substance. Sterilization doses, for convenience, usually are expressed in kilogray (Kgy).

Many investigators have studied the relative resistance of microorganisms to sterilization by radiation. The consensus is that vegetative forms are most sensitive, followed by molds, yeasts, viruses and spore-formers. While past practice has been to use 15 to 25 kGy, today the radiation dose is more closely tailored to the bioburden. It is not unusual to use doses as low as 2 to 8 kGy. The use of the AAMI guidelines or the soon-to-be-published ISO Guidelines are highly recommended.

Modern gamma sterilization facilities used by pharmaceutical and medical device firms generally hold up to 4 MCi of $Cobalt^{60}$. The largest facility holds 12 Mci. Figure 19 shows a schematic of a modern $Cobalt^{60}$ radiosterilization facility.

Two types of electron accelerators are used in sterilization: alternating-current machines with ranges up to 50 kW of power and 5 to 12 meV of energy, and direct-current machines with ranges of 30 to 200 kW and 0.5 to 5 mev. These machines generate electrons at high voltage, accelerate the electrons and spray them on to the product to be sterilized. The greater the machine power (kW), the more electrons can be generated per unit time. The higher the energy (meV), the greater the penetration of the electron into the material to be sterilized.

Ultraviolet (UV) Radiation

Artifically produced UV radiation in the region of 253.7 nm has been used as a germicide for many years. While UV radiation often is used in the pharmaceutical industry for the maintenance of aseptic areas and rooms, it is of limited value as a sterilizing agent.

Inactivation of microorganisms by UV radiation is principally a function of the radiant energy dose, which varies widely for different microorganisms. The primary mechanism of microbial inactivation is the creation of the thymidine dimers in DNA which prevents replication. Vegetative bacteria are most susceptible, while bacterial spores appear to be 3 to 10 times as resistant to inactivation and fungal spores may be 100 to 1000 times more resistant. Bacterial spores on stainless-steel surfaces require approximately 800 μw min/cm^2 for inactivation. By comparison, the black spores of Aspergillus niger require and exposure of over 5000 μW min/cm^2. Even with an adequate dose, however, the requirements for proper application of germicidal UV radiation in most pharmaceutical situations are such as to discourage its use for *sterilization* purposes. On the other hand, as an ancillary germicidal agent, UV radiation can be useful.

When using UV radiation, it is very important that lamps be cleaned periodically with alcohol and tested for output: also its use requires that personnel be properly protected; eye protection is particularly important.

The principal disadvantage to the use of germicidal UV radiation is its limited penetration—its 253.7 nm wavelength is screened out by most materials, allowing clumps of organisms, and those protected by dust or debris, to escape the lethal action. The use of UV radiation as a sterilizing agent is not recommended unless the material to be irradiated is very clean and free of crevices that can protect microorganisms. Many organisms are capable of repairing the UV-induced DNA damage using photoreactivation (light repair) and dark repair.

Aseptic Processing

Although not actually a sterilization process, aseptic processing is a technique frequently used in the compounding of prescriptions or commercial products that will not withstand sterilization but in which all of the ingredients are sterile. In such cases, sterility must be maintained by using sterile materials and a controlled working environment. All containers and apparatus used should be sterilized by one of the previously mentioned processes and such work should be conducted only by an operator fully versed in the control of contamination. The use of laminar-airflow devices or barrier technology for aseptic processing is essential.

With the availability of sterile bulk drugs and sterilized syringe parts from manufacturers, the purchase of several pieces of equipment permits pharmacies to produce filled sterile unit-dose syringes with minimum effort. The equipment needs have been described in a paper by Patel *et al.*[19] Figure 24[19] illustrates this system.

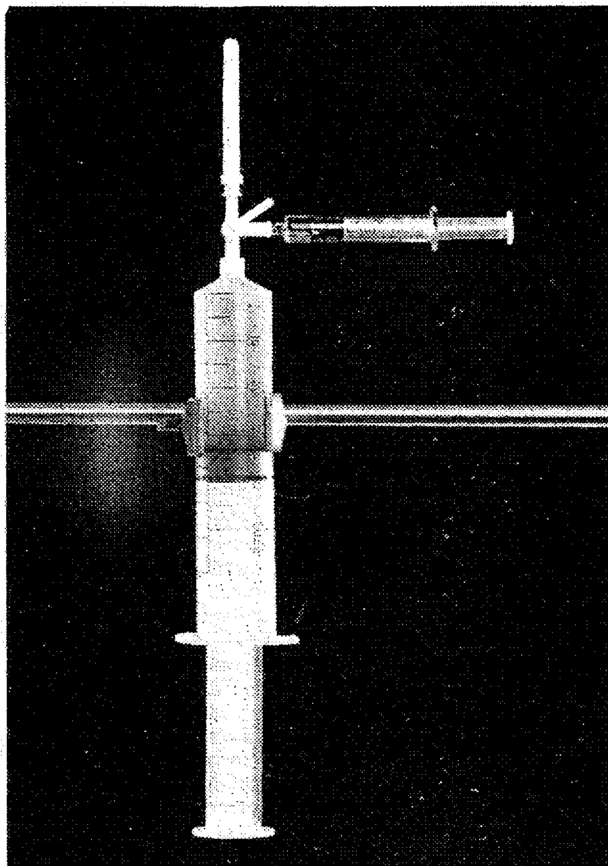


Fig 24. Unit-of-use system for sterile injectable medication.[19]

Packaging

Following exposure of a product to a well-controlled sterilization treatment, the packaging material of the product is expected to maintain sterility until the time of use. Packaging must be durable, provide for permanent-seal integrity and have pore sizes small enough to prevent entry of contaminants. Obviously, the packaging must be compatible with the method of sterilization.

The package design is important if the contents are to be removed without recontamination. Tearing of plastics or paper can be tempered by coatings, and sealed containers should be tested carefully to assure retention of sterility at the time of use.

If sterile material passes through many hands, it is important to provide a tamperproof closure to indicate if the container has been opened inadvertently. These four features—compatibility with sterilization, proven storage protection, ease of opening, tamperproofing—are highly desirable characteristics of medical packaging.

For hospitals and pharmacies, there are a wide variety of woven reusable materials or nonwoven disposable materials which provide acceptable sterile barriers and are offered by major packaging suppliers. These suppliers normally conduct extensive programs to assure the ability of the material to maintain sterility. Both hospitals and industry have guidelines and accepted practices for sterile-product packaging.[5]

A review of the principles of sterile-material packaging by Powell[20] discusses the suitability of packaging materials for various sterilization methods, including resistance to bacteria, types of openings, strength of packaging, testing of packaging and types of packaging. These topics also are discussed in Chapter 85.

Laminar Airflow

Laminar-airflow equipment is essential for proper performance of sterility tests and aseptic filling or assembling operations. These procedures require exact control over the working environment, but while many techniques and different types of equipment for performing these operations have been used over the years, laminar-airflow devices are superior to all other environmental controls.

The laminar-airflow procedure for producing very clean and dust-free areas was developed in 1961. In a laminar-airflow device the entire body of air within a confined area moves with uniform velocity along parallel flow lines. By employing prefilters and high-efficiency bacterial filters, the air delivered to the area essentially is sterile and sweeps all dust and airborne particles from the chamber through an open side. The velocity of the air used in such devices is generally 90 fpm ± 20%. Laminar-airflow devices that deliver the clean air in a vertical, horizontal or curvilinear fashion are available. The devices can be in the form of rooms, cabinets or benches. For a comprehensive discussion of the biomedical application of laminar airflow the reader is referred to Runkle and Phillips.[21]

Each laminar-airflow cabinet or bench should be located in a separate, small, clean room having a filtered air supply.

The selection of the type of cabinet will depend on the operation itself. For most sterility-testing operations, horizontal laminar-airflow units appear to be superior to vertical-flow hoods because the air movement is less likely to wash organisms from the operator's hands or equipment into the sterility test media. Figure 25 shows the sterility testing of syringes in a horizontal laminar airflow hood. Figure 26 shows the design of a typical horizontal, laminar-airflow hood. The major disadvantage of the horizontal laminar-airflow units is that any airborne particulate matter generated in the units is blown directly into the room and against the working personnel. In situations where infectious material is involved, or where one must prevent contamination of the environment with a powder or drug, the use of specifically designed vertical, recirculating laminar-flow units is recommended. Units are available that do an excellent job of providing both product and personnel protection. Such a unit is shown in Fig 27.

To achieve maximum benefit from laminar airflow, it is important first to realize that the filtered airflow does not itself remove microbial contamination from the surface of objects. Thus, to avoid product or test contamination, it is necessary to reduce the microbial load on the outside of materials used in sterility testing. Laminar flow will do an excellent job of maintaining the sterility of an article bathed in the airflow; however, to be accurate, the sterility-testing, or product-assembly procedure must create the least possible turbulence within the unit. Moreover, an awareness of the turbulent air patterns created by the operation is necessary to avoid performing critical operations in turbulent zones. To illustrate how effectively airborne particles are washed from an environment by laminar airflow, Fig 28 shows the distance various-size particles will travel horizontally before falling 5 ft in a cross-flow of air moving at 50 fpm.

Laminar-flow clean benches should supply Class 100 air as defined in Federal Standard 209B.[22] They should be certified to this standard when installed and then tested periodically An air velometer should be used at regular intervals to check the airflow rates across the face of the filter. Smoke tests are useful in visualizing airflow patterns and a particle analyzer can be used to check the quality of the air. The hot dioctyl phthalate (DOP) test generally is employed to check filter efficiency. This standard acceptance test determines the validity of the filter and its seal using DOP smoke (mean particulate diameter of 0.3 μm) and a light-scattering aerosol photometer. The smoke, at a concentration of 80 to 100 mg/L, is introduced to the plenum of the unit and the entire perimeter of the filter face is scanned with the photometer probe at a sampling rate of 1 ft^3/min. A reading of 0.01% of the upstream smoke concentration is considered a leak.

In addition to the routine airflow measurements and filter-efficiency testing, biological testing should be done to monitor the effectiveness of laminar-airflow systems. Microbial air sampling and agar-settling plates are useful in monitoring

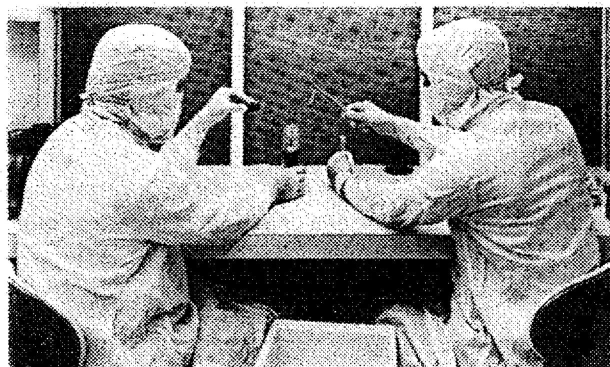


Fig 25. Serility testing of plastic disposable syringes in a horizontal laminar-airflow bench (courtesy, Becton Dickinson & Co).

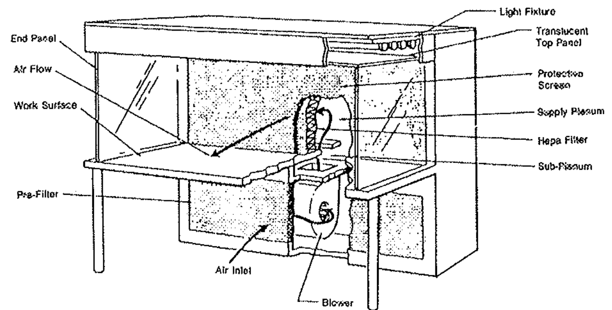


Fig 26. Horizontal laminar-airflow hood.

these environments. Phillips evaluated horizontal laminar-flow hoods by tabulating the number of "false positives" appearing in sterility-test media over a period of time. These results (Table 2) showed very low numbers of "false positives."

Testing

After sterilization, there are several techniques for determining mining whether or not the particular lot of material is sterile. The only method for determining sterility with 100% assurance would be to run a total sterility test, ie, to test every item in the lot.

Representative probabilities are shown in Tables 3 and 4 to illustrate more specifically how low levels of contamination in treated lots of medical articles may escape detection by the usual sterility-test procedures. The data are calculated by binomial expansion, employing certain assumed values of percent contamination with large lot sizes (greater than 5000)

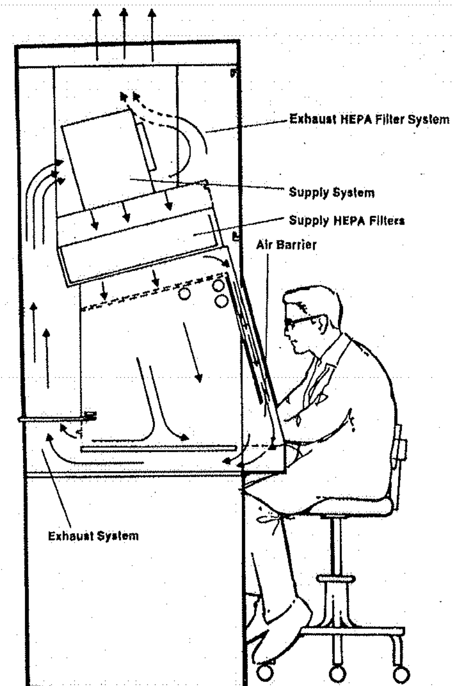


Fig 27. Sketch (*above*) of a biological cabinet with vertical, recirculating laminar-airflow and HEPA-filtered exhaust. HEPA-filtered air is supplied to the work area at 90 fpm ± 20%. Airflow patterns in combination with a high-velocity curtain of air form a barrier at the front access opening which protects both the work and the worker from airborne contamination (courtesy, Bioquest).

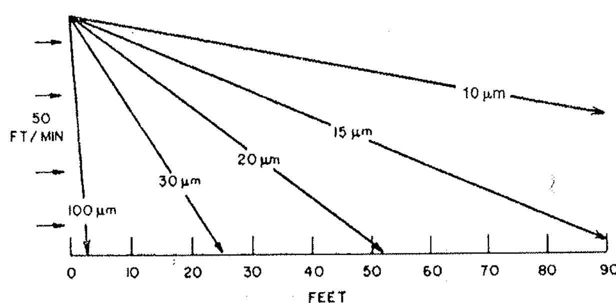


Fig 28. Distance traveled by particles settling from a height of 5 ft.

and including standard assumptions with regard to the efficiency of recovery media, etc.

In Table 3 the probability data are calculated for lots with various degrees of assumed contamination when 10 random samples per lot are tested. For example, a lot that has one in each 1000 items contaminated (0.1% contamination) could be passed as satisfactory (by showing no positive samples from 10 tested) in 99 tests out of 100. Even at the 10% contamination level, contamination would be detected only two out of three times.

Table 4 shows the difficulty in attempting to improve the reliability of sterility tests by increasing sample size. For contamination levels as low as 0.1%, increasing the sample size from 10 to 100 has a relatively small effect in improving the probability of accepting lots. Even a sample size of 500 would result in erroneously accepting a lot six times out of ten. On the other hand, with a lot contaminated to the extent of 10%, by testing 100 samples the probability of acceptance of the lot would be reduced to a theoretical zero.

The information in Table 4 may be viewed in another way. If, for the probability values shown for each different sample size, the value that approximates the 95% confidence level (P = 0.05) is selected, it is clear that using 20 samples only will discriminate contamination levels of 15% or more. If the 20 tubes show no growth the lot could, of course, be sterile but there would be no way of knowing this from the test. From such a test it could be stated only that it is unlikely that the lot would be contaminated at a level higher than 15%. It is clear from these data that product sterility testing is a poor method of validating sterilization procedures.

The USP provides two basic methods for sterility testing. One involves the direct introduction of product test samples into culture media; the second involves filtering test samples through membrane filters, washing the filters with fluids to remove inhibitory properties and transferring the membrane aseptically to appropriate culture media. Test samples may be sterilized devices that simply are immersed aseptically into the appropriate culture-broth washings of the sterile object with sterile diluent, or dilutions of sterile materials. The USP recommends three aqueous diluting fluids for sterility tests while the Antibiotic Regulations list four; all are nontoxic to microorganisms. In the case of petrolatum-based drugs, a nonaqueous diluting fluid is required.

Many studies have been conducted to find the minimum number of culture media that will provide the greatest sensitivity in detecting contamination. Internationally recognized experts and bodies now recommend the use of two culture media: Soybean-Casein Digest Medium, incubated at 20 to 25°, and Fluid Thioglycollate Medium, incubated at 30 to 35°. The time of incubation specified usually is 7 days for the membrane filtration method and 7 to 14 days for the direct-inoculation method, depending on the method of sterilization. The requirements are described in detail in the USP.

The preferred method of verifying sterility is not by testing sterilized materials but by the use of biological indicators. This is not possible, however, when products are sterilized by filtration and filled aseptically into their final containers, as is the case with such important drugs as antibiotics, insulin or hormones. The indicators generally are highly resistant bacterial spores present in greater numbers than the normal contamination of the product and with equal or greater resistance than normal microbial flora in the products being sterilized. Various properties of commercially available bacterial spores have been recommended for specific methods of sterilization based on unique resistance characteristics. Commonly accepted species of bacteria used for biological indicators are shown in Table 5. Other species can be employed, probably without serious impact on the validity of sterility interpretation, so long as the prime requirements of greater numbers and higher resistance, compared to material contamination characteristics, are maintained.

Included with the materials being sterilized, biological indicators are imbedded on either paper or plastic strips or are inoculated directly onto the material being sterilized. Obviously, the indicator has greater validity in verifying sterility if it is located within product spaces that are the most difficult to sterilize. For example, in the case of a syringe, the location of a paper strip or inoculation of spores between the ribs of the plunger stopper is recommended.

Table 2—False Positives Occurring in a Laminar-Flow Hood[26]

Product	No. of units sterility tested	No. of false positives	% false positives
Syringes	9793	2	0.02
Needles	4676	2	0.04
Misc	306	0	0

Table 3—Probabilities for Sterility Testing of Articles with Assumed Levels of Contamination

"True" % contamination	Probability of designated positives out of 10 samples tested			
	0	1	5	10
0.1	0.990	(Total = 0.010)		
1.0	0.904	0.091		
5.0	0.599	0.315		
10.0	0.349	0.387	0.001	
30.0	0.028	0.121	0.103	
50.0	0.001	0.010	0.246	0.001

Table 4—Relationship of Probabilities of Acceptance of Lots of Varying Assumed Degrees of Contamination to Sample Size

Number of samples tested (n)	Probability of no positive growth "True" % contamination of lot					
	0.1	1	5	10	15	20
10	0.99	0.91	0.60	0.35	0.20	0.11
20	0.98	0.82	0.36	0.12	0.04	0.01
50	0.95	0.61	0.08	0.007		
100	0.91	0.37	0.01	0.00		
300	0.74	0.05				
500	0.61	0.01				

Table 5—Species of Bacteria Used as Biological Indicators

Method of sterilization	Bacterial species
Moist heat	*B stearothermophilus*
Dry heat	*B subtilis*
Ethylene oxide	*B stearothermophilus*
Radiation	*B pumilus, B stearothermophilus, B subtilis*

The use of isolators (barrier technology) for processing materials is discussed under *Advanced Aseptic Processing*. The first widespread use of these modern "glove boxes" in the pharmaceutical industry was in sterility testing. As filling speeds became faster, batches became larger. This, coupled with more expensive drug substances, created the need to avoid false-positive sterility tests. Even with laminar-flow hoods becoming widely used, the large number of manipulations carried out by people, created a significant chance for contamination through the testing procedure.

Government standards for SAL basically eliminated the possibility to repeat sterility tests. This means that batches which fail for any reason cannot be released. They are only useful for investigation of potential contamination hazards. Industry in turn needed more assurance that the product was indeed not sterile and the test was valid. This led to the development of more sophisticated isolation units. Figure 29 shows a stainless-steel half-suit system, which is typical of those used in sterility testing. The units can be "docked" to a sterilizer, which eliminates the possibility of contamination during transfer of materials to the test area. The units can be sterilized using vaporized hydrogen peroxide. The exterior of any test materials required to be transferred into the units also can be sterilized in this manner. Validation of these steps allows one to virtually eliminate false-positive test results. Most manufacturers have adopted this technique and have agreed to a policy of essentially no sterility retests. Only where obvious system breakdowns can be shown to have led to failures will a retest be considered.

Advanced Aseptic Processing—Isolator technology also is being used with increasing frequency in the processing of sterile products and associated packaging materials. This is driven by the same need to minimize human intervention and thus increase dramatically the assurance of sterility (SAL). The minimization of people was expanded throughout the 1980s with the advent of more widespread use of form, fill and seal technology. This involved the molding, filling and sealing of plastic bottles, containing sterile products, on-line. The technology was housed in rigid walled areas and product was supplied through filters and sterilized in place, at the last possible area before filling.

While form, fill and seal is a technology on its own, it did lead to the recognition that by updating significantly the older concepts of "glove boxes," one could dramatically affect the sterility assurance of an aseptic process. People contribute the largest percentage of the contamination risk. By minimizing their interaction the probability of nonsterility is greatly reduced.

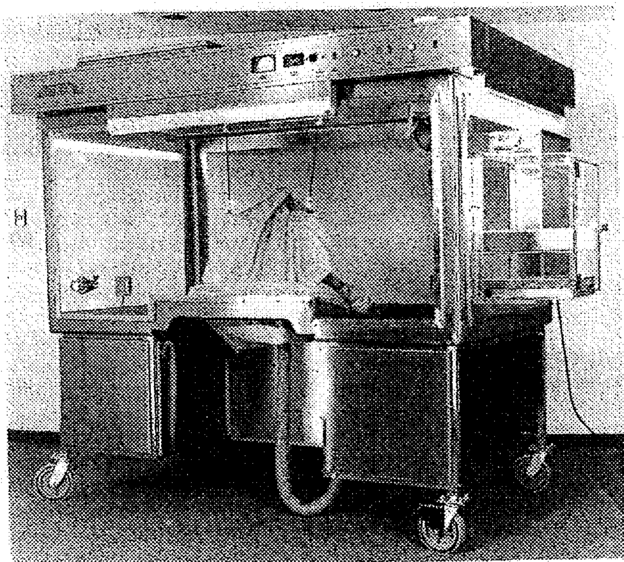


Fig 29. Stainless-steel half-suit isolator (courtesy, Laminar Flow).

Glove boxes were not designed to support modern (and especially more automated) operations. This may explain why they did not become popular as aseptic processing units. It was only when the need for increased assurance drove designers to develop ergonomically designed isolators that the usage began to expand.

More recently, the manufacturers of high-speed processing equipment have begun to redesign their machinery in line with the principles of isolator units. Since the mechanics of these machines have been proved to be very reliable and require very little human intervention, the timing seems to be correct for such modifications. Isolator units are relatively inexpensive also. They have allowed for aseptic processing without the construction of large processing areas, sterile suites or gowning areas. The development of relatively safe sterilization methods, such as vaporized hydrogen peroxide (with or without atmospheric steam) also has allowed the technology to become more viable for widespread use. Prior to this, the use of toxic (and sometimes corrosive) materials was required. This limited their use to more sophisticated operators, who were able to afford the resources required to build and maintain facilities for their use.

Training

It is desirable that personnel involved with sterilization or aseptic processing be instructed in the basic behavior of microorganisms. This would include the differentiation of vegetative, spore-forming and slow-growing life forms such as molds and yeasts. This would allow those being trained in the operations to understand the reasons for many of the restrictions necessary to carry out these processes.

It is imperative that each person involved in these operations be instructed in two main areas.

Safety is the first and foremost area of concentration for a training program. Each of the pieces of equipment and processes described above have unique hazards associated with them. The operators must be made to understand the dangers of steam under pressure and exposure to gaseous sterilants, prior to their neutralization.

The equipment design and installation should undergo safety reviews prior to its general operation. This review for potential hazards must be done by highly trained individuals and should include computer control and piping systems. It is important that the equipment fail (should a failure occur) in a manner which is safe to the operators. Valves should fail in a way to vent pressure to some safe area and/or gases to a relatively safe, unoccupied site.

The second major area of training involves that of gowning for entry into the sterile areas and subsequent performance of aseptic operations. Personnel must be instructed in proper gowning techniques, so that they do not contaminate the exterior of garments and gloves during the process. Gowning areas should be supplied with full length mirrors so that personnel can verify that all areas of their body have been covered fully and properly prior to entering a sterile work area. Recent trends indicate that gowning training be followed by personnel monitoring with contact plates containing growth media. This allows one to verify the effectiveness of the training and, should growth occur, one can use this growth as a training tool to emphasize the importance of careful attention to detail during the gowning process. Since these plates require incubation, one does not allow operators to enter the sterile area until the results of these tests have been collected and reviewed with the candidate.

Continuing with the above approach, those performing aseptic operations require additional training and subsequent verification. This principle of competency-based training (ie, verifying the capabilities of those being trained) is necessary to assure that the operators have developed the skills to carry out these vital operations while minimizing the risk of contamination. Again, it allows for constructive feedback to those who have not yet become fully accomplished in the techniques. It is prudent to reinforce these skills periodically through refresher sessions, and reverification of the skills. It has become standard practice to do unannounced spot-checks of the gloves and gowns of aseptic operators. This practice helps to maintain a level of vigilance, with regard to proper gowning and operating technique.

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CHAPTER 87

Parenteral Preparations

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The distinctive characteristics of parenteral (Gk, *para enteron,* beside the intestine) dosage forms of drugs will be discussed in this chapter. These dosage forms differ from all other drug dosage forms because of the unique requirements imposed because they are injected directly into body tissue through the primary protective system of the human body, the skin and mucous membranes. Therefore, they must be exceptionally pure and free from physical, chemical and biological contaminants. These requirements place a heavy responsibility on the pharmaceutical industry to practice good manufacturing practices (GMPs) in the manufacture of parenteral dosage forms and upon pharmacists to practice good aseptic practices (GAPs) in dispensing them for administration to patients.

Many of the newer drugs, particularly these derived from the new developments in biotechnology, can only be given parenterally because they are inactivated in the gastrointestinal tract, when given by mouth. Further, the potency and specificity of many of these drugs requires strict control of their administration to the patient. A parenteral route of administration meets both of these critical requirements.

This chapter will begin with a brief review of the historical events contributing to the development of this distinctive dosage form. Consideration will then be given to some of the distinguishing characteristics of these dosage forms and how they are administered to patients. The majority of the remainder of the chapter will discuss the various factors required for the preparation of a pure, safe and effective parenteral product.

History[1]

One of the most significant events in the beginnings of parenteral therapy was the first recorded injection of drugs into the veins of living animals, in about 1657, by the architect Sir Christopher Wren. From such a very crude beginning, the technique for intravenous injection and knowledge of the implications therefore developed slowly during the next century and a half. In 1855 Dr Alexander Wood of Edinburgh described what was probably the first subcutaneous injection of drugs for therapeutic purposes using a true hypodermic syringe.

The latter half of the 19th century brought increasing concern for safety in the administration of parenteral solutions, largely because of the work of Robert Koch and Luis Pasteur. While Charles Chamberland was developing both hot-air and steam sterilization techniques and the first bacteria-retaining filter (made of unglazed porcelain), Stanislaus Limousin was developing a suitable container, the all-glass ampul. In the middle 1920s Dr Florence Seibert provided proof that the disturbing chills and fever which often followed the intravenous injection of drugs was caused by potent products of microbial growth, pyrogens, which could be eliminated from water by distillation and from glassware by heating at elevated temperatures.

Of the technical developments that have contributed to the high quality standards currently achievable in the preparation of parenteral dosage forms, the two that have probably contributed most are the development of HEPA-filtered laminar airflow and the development of membrane microfiltration for solutions. The former made it possible to achieve ultraclean environmental conditions for processing from solutions by filtration both viable and nonviable particles of microbial size and smaller. However, many other developments in recent years have produced an impressive advance in the technology associated with the safe and reliable preparation of parenteral dosage forms. The following list identifies a few of the events which have contributed to that development.

1926—Parenterals were accepted for inclusion in the fifth edition of the *National Formulary.*

1933—The practical application of freeze-drying to clinical materials was accomplished by a team of scientists at the University of Pennsylvania.

1938—The Food, Drug and Cosmetic Act was passed by Congress, establishing the Food and Drug Administration (FDA).

1944—The sterilant ethylene oxide was discovered.

1946—The Parenteral Drug Association was organized.

1961—The concept of laminar airflow was developed by WJ Whitfield.

1962—The FDA was authorized by Congress to establish current good manufacturing practice (CGMP or GMP) regulations.

1965—Total parenteral nutrition (TPN) was developed by SJ Dudrick.

1972—The Limulus Amebocyte Lysate test for pyrogens in parenteral products was developed by JF Cooper.

1974—The concept of validation of processes used in the manufacture of parenteral products was introduced by the FDA.

1977—The principles for clean-in-place (CIP) and steam-in-place (SIP) were introduced.

Early 1980s—Home Health Care emerged as an alternative for patients whose health status permitted release from a hospital to care in the home environment.

1982—Insulin, derived through the new discipline of biotechnology, ushered in the drug class of polypeptides with their inherent stability challenges for parenteral dosage-form development.

1987—Parametric release was accepted by the FDA for selected products terminally sterilized by a validated heat process.

The FDA published *Guideline on Sterile Products Produced by Aseptic Processing*, one of several nonregulatory publications to help industry know what the FDA considers to be acceptable.

Late 1980s—The development of computer capabilities has led to the automation of many process operations and to a revolution in documentation and recordkeeping.

1991—The FDA proposed requiring manufacturers to use a terminal sterilization process when preparing a sterile drug product unless such a process adversely affects the drug product.

Administration

Injections may be classified in six general categories:

1. Solutions ready for injection.
2. Dry, soluble products ready to be combined with a solvent just prior to use.
3. Suspensions ready for injection.
4. Dry, insoluble products ready to be combined with a vehicle just prior to use.
5. Emulsions.
6. Liquid concentrates ready for dilution prior to administration.

These injections may be administered by such routes as intravenous, subcutaneous, intradermal, intramuscular, intra-

articular and intrathecal. The nature of the product will determine the particular route of administration that may be employed. Conversely, the desired route of administration will place requirements on the formulation. For example, suspensions would not be administered directly into the blood stream because of the danger of insoluble particles blocking capillaries. Solutions to be administered subcutaneously require strict attention to tonicity adjustment, otherwise irritation of the plentiful supply of nerve endings in this anatomical area would give rise to pronounced pain. Injections intended for intraocular, intraspinal, intracisternal and intrathecal administration require the highest purity standards because of the sensitivity of tissues encountered to irritant and toxic substances.

When compared with other dosage forms, injections possess select advantages. If immediate physiological action is needed from a drug, it usually can be provided by the intravenous injection of an aqueous solution. Modification of the formulation or another route of injection can be used to slow the onset and prolong the action of the drug. The therapeutic response of a drug is controlled more readily by parenteral administration since the irregularities of intestinal absorption are circumvented. Also, since the drug normally is administered by a professionally trained person, it confidently may be expected that the dose was actually and accurately administered. Drugs can be administered parenterally when they cannot be given orally because of the unconscious or uncooperative state of the patient, or because of inactivation or lack of absorption in the intestinal tract. Among the disadvantages of this dosage form are the requirement of asepsis at administration, the risk of tissue toxicity from local irritation, the real or psychological pain factor and the difficulty in correcting an error, should one be made. In the latter situation, unless a direct pharmacological antagonist is immediately available, correction of an error may be impossible. One other disadvantage is that daily or frequent administration poses difficulties, either for the patient to visit a professionally trained person or to learn to inject oneself. However, the advent of home health care as an alternative to extended institutional care has mandated the development of programs for training lay persons to administer these dosage forms.

Parenteral Combinations

During the administration of large-volume parenterals (LVPs), such as 1000-mL of 0.9% sodium chloride solution, it is common practice for a physician to order the addition of a small-volume therapeutic parenteral (SVP), such as an antibiotic, to avoid the discomfort for the patient of a separate injection. While the pharmacist is the most qualified health professional to be responsible to prepare such combinations, as is clearly stated in the Hospital Accreditation Manual of the Joint Commission on Accreditation of Healthcare Organizations,[2] interactions among the combined products can be troublesome even for the pharmacist. In fact, incompatibilities can occur and cause inactivation of one or more ingredients or other undesired reactions. In some instances incompatibilities are visible as precipitation or color change, but in other instances there may be no visible effect.

The many potential combinations present a complex situation even for the pharmacist. To aid in making decisions concerning potential problems, a valuable compilation of relevant data has been assembled by Trissel,[3] and is regularly updated. Further, the advent of computerized data storage and retrieval systems has provided a means to organize and gain rapid access to such information. Further information on this subject may be found in Chapter 88.

As studies have been undertaken and more information has been gained, it has been shown that knowledge of variable factors such as pH and the ionic character of the active constituents aids substantially in understanding and predicting potential incompatibilities. Kinetic studies of reaction rates may be used to describe or predict the extent of degradation. Ultimately, a thorough study should be undertaken of each therapeutic agent in combination with other drugs and IV fluids, not only of generic but of commercial preparations, from the physical, chemical and therapeutic aspects.

Ideally, no parenteral combination should be administered unless it has been studied thoroughly to determine its effect on the therapeutic value and the safety of the combination. However, such an ideal situation may not exist. Nevertheless, it is the responsibility of the pharmacist to be as familiar as possible with the physical, chemical and therapeutic aspects of parenteral combinations and to exercise the best possible judgment as to whether or not the specific combination extemporaneously prescribed is suitable for use in a patient.

General Considerations

An inherent requirement for parenteral preparations is that they be of the very best quality and provide the maximum safety for the patient. Therefore, whether they are prepared from commercially available sterile components, as is usually the case in hospital pharmacies and similar sites, or from nonsterile ingredients in a manufacturing mode, as is the case in the pharmaceutical industry, the persons responsible for their preparation must apply their skills intelligently and diligently. Further, the possession and application of high moral and professional ethics on the part of the persons responsible is the ingredient most vital to achieving the desired quality in the products prepared.

The preparation of parenteral products from sterile components in pharmacies of hospitals and similar sites is discussed further in Chapter 88. In this chapter emphasis will be placed on the preparation of parenteral products from non-sterile components in the highly technologically advanced plants of the pharmaceutical industry, using GMP principles. In the pursuit of GMP, consideration should be given to:

1. Ensure the personnel responsible for assigned duties are capable and qualified to perform them.
2. Ensure that ingredients used in compounding the product have the required identity, quality and purity.
3. Validate critical processes to be sure that the equipment used and the processes followed will ensure that the finished product will have the qualities expected.
4. Maintain a production environment suitable for performing the critical processes required, addressing such matters as orderliness, cleanliness and asepsis.
5. Confirm through adequate quality-control procedures that the finished products have the required potency, purity and quality.
6. Establish through appropriate stability evaluation that the drug products will retain their intended potency, purity and quality until the established expiration date.
7. Ensure that processes are always carried out in accord with established, written procedures.
8. Provide adequate conditions and procedures for the prevention of mixups.
9. Establish adequate procedures, with supporting documentation, for investigating and correcting failures or problems in production or quality control.
10. Provide adequate separation of quality-control responsibilities from those of production to assure independent decision making.

The pursuit of GMP is an ongoing effort which must flex with new technological developments and new understanding of existing principles. Because of the extreme importance of quality in health care of the public, the US Congress has given the responsiblity of regulatory scrutiny over the manufacture and distribution of drug products to the FDA. Therefore, the operations of the pharmaceutical industry are subject to the oversite of the FDA and, with respect to manufacturing practices, to the application of the CGMPs.[4] These regulations are discussed more fully in Chapter 110.

In concert with the pursuit of GMPs, the pharmaceutical industry has shown initiative and innovation in the extensive technological development and improvement in quality, safety and effectiveness of parenteral dosage forms in recent years. Further, outstanding innovative development in drug-delivery

systems is occurring. These factors have been additive in providing the public with outstanding parenteral dosage forms of drugs at this time in history.

General Manufacturing Process

The preparation of a parenteral product may be considered to encompass four general areas as follows:

1. Procurement and selection o the components and containers.
2. Production facilities and procedures.
3. Control of quality.
4. Packaging and labeling.

These components of the product to be procured include vehicles, solutes, containers and closures. The steps constituting production include maintaining facilities and equipment, preparing and controlling the environment, cleaning the containers and equipment, preparing the product, filtering the solution, filling containers with the product, sealing the containers and sterilizing the product. The control of quality includes the evaluation of the components, qualification of equipment, validation of processes, determination that the production has been executed within prescribed requirements and performance of necessary evaluative tests on the finished product. The final area of packaging and labeling includes all steps necessary to identify the finished product and enclose it in such manner that it is safely and properly prepared for sale and delivery to the user. The remainder of this chapter will be organized in accord with these four general areas, with emphasis on the first two areas.

Components and Containers

Establishing specifications to ensure the quality of each of the components of an injection is essential. These specifications will be coordinated with the requirements of the specific formulation and will not necessarily be identical for a particular component if used in several different formulations. For example, particle-size control may be necessary for powders used in formulating a suspension but be relatively unimportant for preparing a solution.

The most stringent chemical-purity requirements normally will be encountered with aqueous solutions, particularly if the product is to be sterilized at an elevated temperature where reaction rates will be accelerated greatly. Modification of aqueous vehicles to include a glycol, for example, usually will reduce reaction rates. Dry preparations pose relatively few reaction problems but may require definitive physical specifications for ingredients that must have certain solution or dispersion characteristics when a vehicle is added.

Containers and closures are in prolonged, intimate contact with the product and may release substances into or remove ingredients from the product. Assessment and selection of containers and closures is a necessary part of product formulation to ensure that the product retains its purity, potency and quality during the intimate contact with the container throughout its shelf-life. Administration devices that come in contact with the product should be assessed and selected with the same care as are containers and closures, even though the contact period is usually brief.

Vehicles

Since most liquid injections are quite dilute, the component present in the highest proportion is the vehicle. A vehicle normally has no therapeutic activity and is nontoxic. However, it is of great importance in the formulation since it presents to body tissues the form of the active constituent for absorption. Absorption normally occurs most rapidly and completely when a drug is presented as an aqueous solution. Modification of the vehicle with water-miscible liquids or substitution with water-immiscible liquids normally decreases the rate of absorption. Absorption from a suspension may be affected by such factors as the viscosity of the vehicle, its capacity for wetting the solid particles, the solubility equilibrium produced by the vehicle and the distribution coefficient between the vehicle and aqueous body systems.

The vehicle of greatest importance for parenteral products is water. Water of suitable quality for compounding and rinsing product contact surfaces may be prepared either by distillation or by reverse osmosis, to meet USP specifications for Water for Injection (WFI). Only by these two methods is it possible to separate adequately various liquid, gas and solid contaminating substances from water. These two methods for preparation of WFI will be discussed in this Chapter. It should be noted that there is no unit operation more important and none more costly to install and operate than the one for the preparation of WFI.

Preparation of Water for Injection (WFI)

The source water can be expected to be contaminated with natural suspended mineral and organic substances, dissolved mineral salts, colloidal silicates and industrial chemicals. The degree of contamination will vary with the source and will be markedly different whether obtained from a well or from surface sources, such as a stream or lake. Therefore, this water normally is not of sufficient purity to prepare WFI directly. Hence, the source water usually must be pretreated by one or a combination of the following treatments: chemical softening, filtration, deionization, carbon adsorption or reverse osmosis purification. Space does not permit discussion of these processes here, but the interested reader is referred elsewhere for this information.[5,6]

In general, a conventional still consists of a boiler (evaporator) containing feed water (distilland); a source of heat to vaporize the water in the evaporator; a headspace above the level of distilland with condensing surfaces for refluxing the vapor, thereby returning nonvolatile impurities to the distilland, a means for eliminating volatile impurities before the hot water vapor is condensed; and a condenser for removing the heat of vaporization, thereby converting the water vapor to a liquid distillate.

The specific construction features of a still and the process specifications markedly will affect the quality of distillate obtained from a still. Those required for producing high-purity water, such as WFI, must be considerably more stringent than those required for Purified Water USP. Among the factors that must be considered are:

1. The quality of the feed water will affect the quality of the distillate. Controlling the quality of the feed water is essential for meeting the required specifications for the distillate.
2. The size of the evaporator will affect the efficiency. It should be large enough to provide a low vapor velocity, thus reducing the entrainment of the distilland either as a film on vapor bubbles or as separate droplets.
3. The baffles (condensing surfaces) determine the effectiveness of refluxing. They should be designed to remove efficiently the entrainment at optimal vapor velocity, collecting and returning the heavier droplets contaminated with the distilland.
4. Redissolving volatile impurities in the distillate reduces its purity. Therefore, they should be separated efficiently from the hot water vapor and eliminated by aspirating them to the drain or venting them to the atmosphere.
5. Contamination of the vapor and distillate from the metal parts of the still can occur. Present standards for high-purity stills are that all parts contacted by the vapor or distillate should be constructed of metal coated with pure tin, 304 or 316 stainless steel or chemically resistant glass.

The design features of a still also influence its efficiency of operation, relative freedom from maintenance problems or extent of automatic operation. Stills may be constructed of varying size, rated according to the volume of distillate that can be produced per hour of operation under optimum conditions. Only stills designed to produce high-purity water may be considered for use in the production of WFI.

Conventional commercial stills designed for the production of high-purity water, such as shown in Fig 1, are available from several suppliers (*AMSCO, Barnstead, Corning, Vaponics*).

Compression Distillation—The vapor-compression still, primarily designed for the production of large volumes of high-purity distillate with low consumption of energy and water, is illustrated diagrammatically in Fig 2. To start, the feed water is heated in the evaporator to boiling. The vapor produced in the tubes is separated from the entrained distilland in the separator and conveyed to a compressor which compresses the vapor and raises its temperature to approximately 107°. It then flows to the steam chest where it condenses on the outer surfaces of the tubes containing the distilland; thereby the vapor is condensed and drawn off as a distillate while giving up its heat to bring the distilland in the tubes to the boiling point.

Vapor-compression stills are available in capacities from 50 to 2800 gal/hr (*Aqua-Chem, Barnstead, Meco*).

Multiple-Effect Stills—The multiple-effect still also is designed to conserve energy and water usage. In principle, it is simply a series of single-effect stills running at differing pressures. A series of up to seven effects may be used, with the first effect operated at the highest pressure and the last effect at atmospheric pressure. See a schematic drawing of a multiple-effect still in Fig. 3. Steam from an external source is used in the first effect to generate steam under pressure from feed water; it is used as the power source to drive the second effect. The steam used to drive the second effect condenses as it gives up its heat of vaporization and forms a distillate. This process continues until the last effect when the steam is at atmospheric pressure and must be condensed in a heat exchanger.

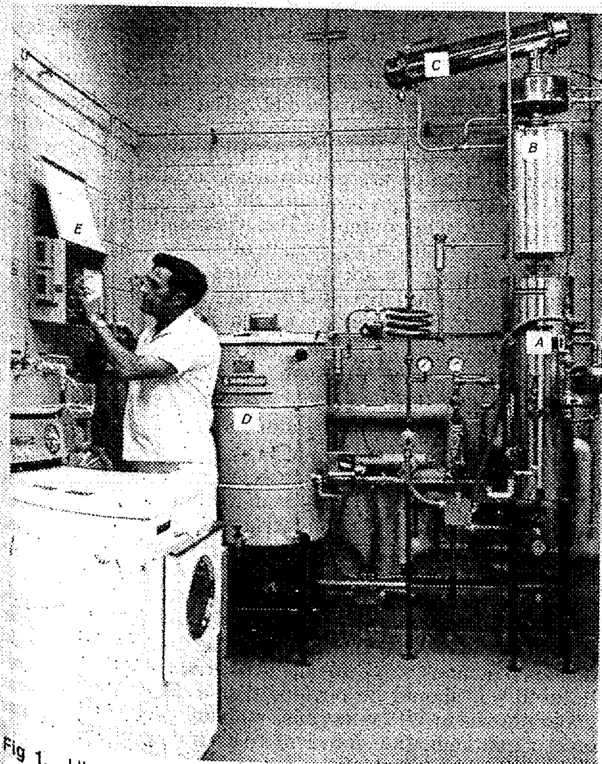


Fig 1. High-purity stilled and sealed water-storage system. *A:* evaporator; *B:* high-purity baffle unit; *C:* condenser; *D:* storage tank with ultraviolet lamp; *E:* control panel (courtesy, Ciba-Geigy).

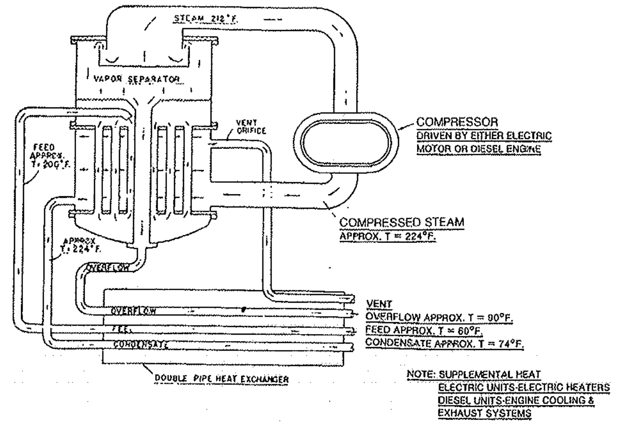


Fig 2. Vapor-compression still.

The capacity of a multiple-effect still can be increased by adding effects. The quantity of the distillate also will be affected by the inlet steam pressure; thus, a 600-gal/hr unit designed to operate at 115 psig steam pressure could be run at approximately 55 psig and would deliver about 400 gal/hr. These stills have no moving parts and operate quietly. They are available in capacities from about 50 to 7000 gal/hr (*AMSCO, Barnstead, Finn-Aqua, Vaponics*).

Reverse Osmosis (RO)—As the name suggests, the natural process of selective permeation of molecules through a semipermeable membrane separating two aqueous solutions of different concentrations is reversed. Pressure, usually between 200 and 400 psig, is applied to overcome osmotic pressure and force pure water to premeate through the membrane. Membranes, usually composed of cellulose esters or polyamides, are selected to provide an efficient rejection of contaminant molecules in raw water. The molecules most difficult to remove are small inorganic ones such as sodium chloride. Passage through two membranes in series is sometimes used to increase the efficiency of removal of these small molecules and to decrease the risk of structural failure of a membrane to remove other contaminants, such as bacteria and pyrogens. For additional information, see *Reverse Osmosis* in Chapter 37 and *Water* in Chapters 80 and 86.

Reverse osmosis systems are available in a range of production sizes. (*AMSCO, Aqua-Chem, Finn-Aqua, Meco, Millipore*, etc).

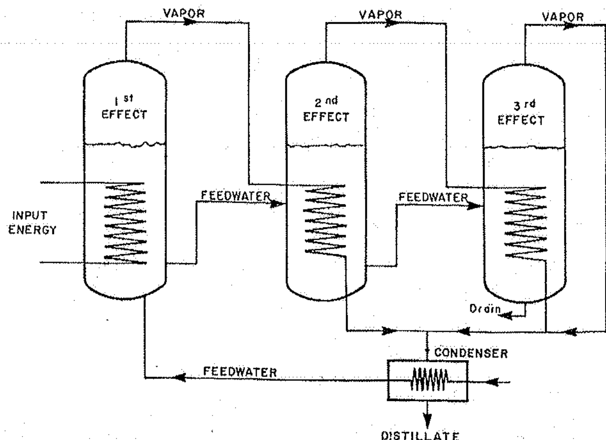


Fig 3. Multiple-effect still. (courtesy, Dekker); taken from Avis KE, Lieberman HA, Lachman L; *Pharmaceutical Dosage Forms: Parenteral Medications*, vol 2, 2nd ed, Dekker, New York, 1993.

Whichever system is used for the preparation of WFI, validation is required to be sure that the system consistently and reliably will produce the chemical, physical and microbiological quality of water required. Such validation should start with the determined characteristics of the source water and include the pretreatment, production, storage and distribution systems. All of these systems together determine the ultimate quality of the WFI. Because of space limitations here, more details concerning the design, operation and validation of these highly important systems may be found in other literature sources.[5,6]

Storage and Distribution—The rate of production of WFI usually is not sufficient to meet processing demands; therefore, it is collected in a holding tank for subsequent use. In large operations the holding tanks may have a capacity of several thousand gallons and be a part of a continuously operating system. In such instances the USP requires that the WFI be held at a temperature too high for microbial growth to occur. Normally, this temperature is a constant 80°.

The USP also permits the WFI to be stored at room temperature, but for a maximum of 24 hours. Under such conditions the WFI usually is collected as a batch for a particular use with any unused water being discarded within 24 hours. Such a system requires frequent sanitization to minimize the risk of viable microorganisms being present. The stainless-steel storage tanks in such systems usually are connected to a welded stainless-steel distribution loop supplying the various use sites with a continuously circulating water supply. The tank is provided with a hydrophobic membrane vent filter capable of excluding bacteria and nonviable particulate matter. Such a vent filter is necessary to permit changes in pressure during filling and emptying. The construction material for the tank and connecting lines is usually electropolished 316L stainless steel with welded pipe. The tanks also may be lined with glass or a coating of pure tin. Such systems are very carefully designed and constructed and often constitute the most costly installation within the plant.

When the water cannot be used at 80°, heat exchangers must be installed to reduce the temperature at the point of use. Bacterial retentive filters should not be installed in such systems because of the risk of bacterial buildup on the filters and the consequential release of pyrogenic substances.

Purity—The USP monographs provide standards of purity for WFI and Sterile Water for Injection (SWFI). A few of these standards require comment.

SWFI must meet the requirements of the USP Sterility Test, but WFI need not since it is to be used in a product which will be sterilized. Both must contain not more than 0.25 Endotoxin units per mL.

The limits for total solids varies in the two monographs. The larger the surface area of the glass container per unit volume of water, the greater the amount of glass constituents that may be leached into the water, particularly during the elevated temperature of steam sterilization. Therefore, the latter treatment will affect the total solids content of SWFI.

The WFL monograph stipulates a maximum of 10 ppm of total solids but SWFI may contain 20 to 40 ppm. This is generally considered to be much too high to assure a quality of water that permits the stable formulation of many drugs. A relatively few metallic ions present often can render a formulation unstable. Therefore, it is common practice to set a limit of 0.1 ppm or less of ionic contaminants expressed as sodium chloride.

Ionic contaminant level is not the same as total solids; the former is a measure of only the ionic content, while the latter is a measure of the undissociated constituents as well. The ionic content of water can be measured very easily by means of a conductivity meter which frequently is used as an indicator of the purity. The results are expressed in one of three terms: as sodium chloride ions, as resistance in ohms or megohms or as conductance in micromhos (or microSiemens). Ohms and mhos have a reciprocal relationship to each other, but they are related to ppm sodium chloride by an experimentally determined curve. The USP is considering introducing a conductivity requirement. To give one point of comparison, 0.1 ppm sodium chloride is equal to approximately 1.01 megohms and 0.99 micromhos. It should be mentioned that conductivity measurements give no direct indication of pyrogen content since pyrogens are undissociated organic compounds.

WFI and SWFI may not contain added substances. Bacteriostatic Water for Injection (BWFI) may contain one or more suitable antimicrobial agents in containers of 30 mL or less. This restriction is designed to prevent the administration of a large quantity of a bacteriostatic agent that probably would be toxic in the accumulated amount of a large volume of solution, even though the concentration was low.

The USP also provides monographs giving the specifications for Sterile Water for Inhalation and Sterile Water for Irrigation. The USP should be consulted for the minor differences between these specifications and those for SWFI.

Types of Vehicles

Aqueous Vehicles—Certain aqueous vehicles are recognized officially because of their valid use in parenterals. Often they are used as isotonic vehicles to which a drug may be added at the time of administration. The additional osmotic effect of the drug may not be enough to produce any discomfort when administered. These vehicles include Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection and Lactated Ringer's Injection.

Water-Miscible Vehicles—A number of solvents that are miscible with water have been used as a portion of the vehicle in the formulation of parenterals. These solvents are used primarily to affect the solubility of certain drugs and to reduce hydrolysis. The most important solvents in this group are ethyl alcohol, polyethylene glycol and propylene glycol. Ethyl alcohol is used particularly in the preparation of solutions of cardiac glycosides and the glycols in solutions of barbiturates, certain alkaloids and certain antibiotics. Such preparations usually are given intramuscularly.

These solvents, as well as nonaqueous vehicles, have been reviewed by Spiegel and Noseworthy.[7]

Nonaqueous Vehicles—The most important group of nonaqueous vehicles are the fixed oils. The USP provides specifications for such vehicles, indicating that the fixed oils must be of vegetable origin so that they will be metabolized, will be liquid at room temperature and will not become rancid readily. The USP also specifies limits for the degree of unsaturation and free fatty acid content. The oils most commonly used are corn oil, cottonseed oil, peanut oil and sesame oil. Fixed oils are used particularly as vehicles for certain hormone preparations. The label must state the name of the vehicle so that the user may beware in case of known sensitivity or other reactions to it.

Solutes

Bulk pharmaceutical chemicals should be prepared under conditions designed to preclude the introduction of chemical, physical and microbiological contamination. To the extent possible, the system of manufacture should be a closed system, particularly if the chemical is to be used in a parenteral formulation. Further, the contact surfaces of all process equipment should be inert to the chemicals being processed. Where the chemicals are to be used in relatively small amounts, marketed commercial grades may be the only forms available. In such instances, the best chemical grade obtainable should be used. It should be obvious that if a few ppm of ionic contaminants in WFI may cause stability problems, a similar level of contamination in the solute itself may, likewise, cause stability problems. Metallic catalysis of chemical reactions is one which is encountered frequently.

Other factors to be considered with respect to the quality of solutes include the level of microbial and pyrogenic contami-

nation, solubility characteristics as determined by the chemical or physical form of the compound and freedom from gross dirt.

Added Substances—The USP includes in this category all substances added to a preparation to improve or safeguard its quality. An added substance may:

Effect solubility, as does sodium benzoate in Caffeine and Sodium Benzoate Injection.

Provide patient comfort, as do substances added to make a solution isotonic.

Enhance the chemical stability of a solution, as do antioxidants, inert gases, chelating agents and buffers.

Protect a preparation against the growth of microorganisms. The term "preservative" sometimes is applied only to those substances which prevent the growth of microoganisms in a preparation. However, such limited use is inappropriate, being better used for all substances that act to retard or prevent the chemical, physical or biological degradation of a preparation.

While added substances may prevent a certain reaction from taking place, they may induce others. Not only may visible incompatibilities occur, but hydrolysis, complexation, oxidation and invisible reactions may decompose or otherwise inactivate the therapeutic agent or other added substances. Therefore, added substances must be selected with due consideration and investigation of their effect on the total formulation.

Antimicrobial Agents—The USP states that antimicrobial agents in bacteriostatic or fungistatic concentrations must be added to preparations contained in multiple-dose containers. They must be present in adequate concentration at the time of use to prevent the multiplication of microorganisms inadvertently introduced into the preparation while withdrawing a portion of the contents with a hypodermic needle and syringe. The USP provides a test for *Antimicrobial Preservative Effectiveness* to determine that an antimicrobial substance or combination adequately inhibits the growth of microorganisms in a parenteral product. Because antimicrobials may have inherent toxicity for the patient, the USP prescribes concentration limits for those that are used commonly in parenteral products, as follows:

Phenylmercuric nitrate and thimerosal 0.01%.
Benzethonium chloride and benzalkonium chloride 0.01%.
Phenol or cresol 0.5%.
Chlorobutanol 0.5%.

The above limit is rarely used for phenylmercuric nitrate, most frequently being employed in a concentration of 0.002%. Methyl *p*-hydroxybenzoate 0.18% and propyl *p*-hydroxybenzoate 0.02% in combination, and benzyl alcohol 2% also are used frequently. In oleaginous preparations, no antibacterial agent commonly employed appears to be effective. However, it has been reported that hexylresorcinol 0.5% and phenylmercuric benzoate 0.1% are moderately bactericidal. A few therapeutic compounds have been shown to have antibacterial activity, thus obviating the need for added agents.

Antimicrobial agents must be studied with respect to compatibility with all other components of the formula. In addition, their activity must be evaluated in the total formula. It is not uncommon to find that a particular agent will be effective in one formulation but ineffective in another. This may be due to the effect of various components of the formula on the biological activity or availability of the compound; for example, the binding and inactivation of esters of *p*-hydroxybenzoic acid by macromolecules such as Polysorbate 80 or the reduction of phenylmercuric nitrate by sulfide residues in rubber closures. A physical reaction encountered is that bacteriostatic agents sometimes are removed from solution by rubber closures.

Single-dose containers and pharmacy bulk packs which do not contain antimicrobial agents are expected to be used promptly after opening or to be discarded. Large-volume single-dose containers may not contain an added antimicrobial preservative. Therefore, special care must be exercised in storing such products after the containers have been opened to prepare an admixture, particularly those that can support the growth of microorganisms, such as total parenteral nutrition (TPN) solutions. It should be noted that while refrigeration slows the growth of most microorganisms, it does not prevent their growth.

Buffers are used primarily to stabilize a solution against the chemical degradation that might occur if the pH changes appreciably. Buffer systems employed should normally have as low a buffer capacity as feasible in order not to disturb significantly the body buffer systems when injected. In addition, the buffer range and effect on the activity of the product must be evaluated carefully. The acid salts most frequently employed as buffers are citrates, acetates and phosphates.

Antioxidants are required frequently to preserve products because of the ease with which many drugs are oxidized. Sodium bisulfite 0.1% is used most frequently. The use of sulfites has been reviewed by Schroeter.[8] Acetone sodium bisulfite, sodium formaldehyde sulfoxylate and thiourea also are used sometimes. The sodium salt of ethylenediaminetetraacetic acid has been found to enhance the activity of antioxidants in some cases, apparently by chelating metallic ions that would otherwise catalyze the oxidation reaction.

Displacing the air (oxygen) in and above the solution by purging with an inert gas, such as nitrogen, also can be used as a means to control oxidation of a sensitive drug. Process control is required to be assured that every container is deaerated adequately and uniformly.

Tonicity Agents are used in many parenteral and ophthalmic products to control the tonicity. However, not all preparations need to be isotonic. The agents most commonly used are electrolytes and mono- or disaccharides. This subject is considered much more extensively in Chapter 36.

Pyrogens (Endotoxins)

Pyrogens are products of metabolism of microorganisms. The most potent pyrogenic substances (endotoxins) are constituents of the cell wall of Gram-negative bacteria. Gram-positive bacteria and fungi also produce pyrogens but of lower potency and of different chemical nature. Endotoxins are high-molecular-weight (about 20,000 daltons) lipopolysaccharides. Studies have shown that the lipid portion of the molecule is responsible for the biological activity. Since endotoxins are the most potent pyrogens and Gram-negative bacteria are ubiquitous in the environment, this discussion will focus on endotoxins and the risk of their presence as contaminants in sterile products.

Pyrogens, when present in parenteral drug products and injected into patients, can cause fever, chills, pain in the back and legs, and malaise. While pyrogenic reactions are rarely fatal, they can cause serious discomfort and, in the seriously ill patient, shock-like symptoms that can be fatal. The intensity of the pyrogenic response and its degree of hazard will be affected by the medical condition of the patient, the potency of the pyrogen, the amount of the pyrogen and the route of administration (intrathecal is most hazardous followed by intravenous, intramuscular and subcutaneous). When bacterial (exogenous) pyrogens are introduced into the body, leucocytic phagocytosis is believed to occur and endogenous pyrogen is produced. The endogenous pyrogen then produces the familiar physiological effects. Space will not permit further elaboration of these matters here, but the reader is referred to the work by Pearson[9] if more information is needed.

Control of Pyrogens—Pyrogens are contaminants if present in parenteral drug products, and should not be there. In general, it is impractical, if not impossible, to remove pyrogens once present without adversely affecting the drug product. Therefore, the emphasis should be on the prevention of the introduction or development of pyrogens in all aspects of the compounding and processing of the product.

Pyrogens may enter a preparation through any means that will introduce living or dead microorganisms. However, current technology generally permits the control of such contamination, and the presence of pyrogens in a finished product is

indicative of processing under inadequately controlled clean conditions. It also should be noted that time for microbial growth to occur increases the risk for elevated levels of pyrogens. Therefore, compounding and manufacturing processes should be carried out as expeditiously as possible, preferably planning completion of the process, including sterilization, within one work day.

Pyrogens can be destroyed by heating at high temperatures. The recommended procedure for depyrogenation of glassware and equipment is heating at a temperature of 250° for 45 minutes. It has been reported that 650° for 1 minute or 180° for 4 hours likewise will destroy pyrogens. The usual autoclaving cycle will not do so. Heating with strong alkali or oxidizing solutions will destroy pyrogens. It has been claimed that thorough washing with detergent will render glassware pyrogen-free if subsequently rinsed thoroughly with pyrogen-free water. Plastic containers and devices must be protected from pyrogenic contamination during manufacture and storage since known ways to destroy pyrogens will affect the plastic adversely. It has been reported that anion-exchange resins and positively charged membrane filters will remove pyrogens from water. Also, reverse osmosis will eliminate them. However, the most reliable method for their elimination from water is distillation.

A method that has been used for the removal of pyrogens from solutions is adsorption on adsorptive agents. However, since the adsorption phenomenon also may cause selective removal of chemical substances from the solution, this method has limited application. Other in-process methods for their destruction or elimination include selective extraction procedures and careful heating with dilute alkali, dilute acid or mild oxidizing agents. In each instance, the method must be studied thoroughly to be sure it will not have an adverse effect on the constituents of the product. Developments in ultrafiltration now make possible pyrogen separation on a molecular weight basis and the process of tangential flow is making large-scale processing a reality.

Sources of Pyrogens—Through understanding the means by which pyrogens may contaminate parenteral products, their control becomes more achievable. Therefore, it is important to know that water is probably the greatest potential source of pyrogenic contamination, since water is essential for the growth of microorganisms. When microorganisms metabolize, pyrogens will be produced. Therefore, raw water can be expected to be pyrogenic and only when it is appropriately treated to render it free from pyrogens, such as WFI, should it be used for compounding the product or rinsing product contact surfaces such as tubing, mixing vessels and rubber closures. Even when such rinsed equipment and supplies are left wet and improperly exposed to the environment, there is a high risk that they will become pyrogenic. As stated previously, although proper distillation will provide pyrogen-free water, storage conditions must be such that microorganisms are not introduced and subsequent growth is prevented.

Other potential sources of contamination are containers and equipment. Pyrogenic materials adhere strongly to glass and other surfaces. Residues of solutions in used equipment often become bacterial cultures with subsequent pyrogenic contamination. Since drying does not destroy pyrogens, they may remain in equipment for long periods. Adequate washing will reduce and subsequent dry-heat treatment can render contaminated equipment suitable for use. However, all such processes must be validated to assure their effectiveness.

Solutes may be a source of pyrogens. For example, the manufacturing of bulk chemicals may involve the use of pyrogenic water for process steps such as crystallization, precipitation or washing. Bulk drug substances derived from fermentation will almost certainly be heavily pyrogenic. Therefore, all lots of solutes used to prepare parenteral products should be tested to ensure that they will not contribute unacceptable quantities of endotoxin to the finished product.

The manufacturing process must be carried out with great care and as rapidly as possible to minimize the risk of microbial contamination. Preferably, no more product should be prepared than can be processed, completely within one working day, including sterilization.

Containers

Containers are an integral part of the formulation of an injection. No container is totally insoluble or does not in some way affect the liquid it contains, particularly if the liquid is aqueous. Therefore, the selection of a container for a particular injection must be based on a consideration of the composition of the container, as well as of the solution, and the treatment to which it will be subjected.

Table 1 provides a generalized comparison of the three compatibility properties—leaching, permeation and adsorption—of container materials most likely to be involved in the formulation of aqueous parenterals. Further, the integrity of the container/closure system depends upon several characteristics, including container opening finish, closure modulus, durometer and compression set, and aluminum seal application force. These considerations have been reviewed by Morton.[10]

Table 1—Comparative Compatibility Properties of Container Materials

	Leaching		Permeation		Adsorption (selective) extent[a]
	Extent[a]	Potential leachables	Extent[a]	Potential agents	
Glass					
Borosilicate	1	Alkaline earth and heavy metal oxides	0	N/A	2
Soda-lime	5	Alkaline earth and heavy metal oxides	0	N/A	2
Plastic polymers					
Polyethylene					
Low density	2	Plasticizers, antioxidants	5	Gases, water vapor, other molecules	2
High density	1	Antioxidants	3	Gases, water vapor, other molecules	2
PVC	4	HCl, especially plasticizers, antioxidants, other stabilizers	5	Gases, especially water vapor and other molecules	2
Polyolefins	2	Antioxidants	2	Gases, water vapor, other molecules	2
Polypropylene	2	Antioxidants, lubricants	4	Gases, water vapor	1
Rubber polymers					
Natural and related synthetic	5	Heavy metal salts, lubricants, reducing agents	3	Gases, water vapor	3
Butyl	3	Heavy metal salts, lubricants, reducing agents	1	Gases, water vapor	2
Silicone	2	Minimal	5	Gases, water vapor	1

[a] Approximate scale of 1 to 5 with "1" as the lowest.

Plastic

Thermoplastic polymers have been established as packaging materials for sterile preparations such as large-volume parenterals, ophthalmic solutions and, increasingly, for small-volume parenterals. For such use to be acceptable a thorough understanding of the characteristics, potential problems and advantages for use must be developed. A historical review of these factors relative to pharmaceuticals has been prepared by Autian.[11] A recent discussion of polymers for IV solutions has been published by Lambert.[12] Autian stated that three principal problem areas exist in using these materials; namely,

1. Permeation of vapors and other molecules in either direction through the wall of the plastic container.
2. Leaching of constituents from the plastic into the product.
3. Sorption (absorption and/or adsorption) of drug molecules or ions on the plastic material.

Permeation, the most extensive problem, may be troublesome by permitting volatile constituents, water or selected drug molecules to migrate through the wall of the container to the outside and thereby be lost. This problem has been resolved, for example, by the use of an overwrap in the packaging of IV solutions in PVC bags to prevent the loss of water during storage. Reverse permeation also may occur by which oxygen or other molecules may penetrate to the inside of the container and cause oxidative or other degradation of susceptible constituents. Leaching may be a problem when certain constituents in the plastic formulation, such as plasticizers or antioxidants, migrate into the product. Thus, plastic polymer formulations should have as few additives as possible, an objective characteristically achievable for most plastics being used for parenteral packaging. *Sorption* is a problem on a selective basis, that is, sorption of a few drug molecules occurs on select polymers. For example, sorption of insulin, vitamin A acetate and warfarin sodium has been shown to occur on PVC bags and tubing when these drugs were present as additives in IV admixtures. A brief summary of some of these compatibility relationships is given in Table 1.

One of the principle advantages of using plastic packaging materials is that they are not breakable as is glass; also, there is a substantial weight reduction. The flexibility of the low-density polyethylene polymer, for ophthalmic preparations, makes it possible to squeeze the side wall of the container and discharge one or more drops without introducing contamination into the remainder of the product. The flexible bags of polyvinyl chloride or select polyolefins, currently in use for large-volume intravenous fluids, have the added advantage that no air interchange is required; the flexible wall simply collapses as the solution flows out of the bag.

Most plastic materials have the disadvantage that they are not as clear as glass and, therefore, inspection of the contents is impeded. In addition, many of these materials will soften or melt under the conditions of thermal sterilization. However, careful selection of the plastic used and control of the autoclave cycle has made thermal sterilization of some products possible, large-volume parenterals in particular. Ethylene oxide or radiation sterilization may be employed for the empty container with subsequent aseptic filling. However, careful evaluation of the residues from ethylene oxide or its degradation products and their potential toxic effect must be undertaken. Investigation is required concerning potential interactions and other problems that may be encountered when a parenteral product is packaged in plastic. For further details see Chapter 85.

Glass

Glass is employed as the container material of choice for most SVPs. It is composed principally of silicon dioxide with varying amounts of other oxides such as sodium, potassium, calcium, magnesium, aluminum, boron and iron. The basic structural network of glass is formed by the silicon oxide tetrahedron. Boric oxide will enter into this structure, but most of the other oxides do not. The latter are only loosely bound, are present in the network interstices and are relatively free to migrate. These migratory oxides may be leached into a solution in contact with the glass, particularly during the increased reactivity of thermal sterilization. The oxides thus dissolved may hydrolyze to raise the pH of the solution, catalyze reactions or enter into reactions. Additionally, some glass compounds will be attacked by solutions and, in time, dislodge glass flakes into the solution. Such occurrences can be minimized by the proper selection of the glass composition.[13]

Types—The USP has aided in this selection by providing a classification of glass; namely,

Type I, a borosilicate glass.
Type II, a soda-lime treated glass.
Type III, a soda-lime glass.
NP, a soda-lime glass not suitable for containers for parenterals.

Type I glass is composed principally of silicon dioxide and boric oxide, with low levels of the non-network-forming oxides. It is a chemically resistant glass (low leachability) also having a low thermal coefficient of expansion.

Types II and III glass compounds are composed of relatively high proportions of sodium oxide and calcium oxide. This makes the glass chemically less resistant. Both types melt at a lower temperature, are easier to mold into various shapes and have a higher thermal coefficient of expansion than Type I. While there is no one standard formulation for glass among manufacturers of these USP type categories, Type II glass usually has a lower concentration of the migratory oxides than Type III. In addition, Type II has been treated under controlled temperature and humidity conditions with sulfur dioxide to dealkalize the interior surface of the container. While it remains intact, this surface will increase substantially the chemical resistance of the glass. However, repeated exposures to sterilization and alkaline detergents will break down this dealkalized surface and expose the underlying soda-lime compound.

The glass types are determined from the results of two USP tests: the Powdered Glass Test and the Water Attack Test. The latter is used only for Type II glass and is performed on the whole container, because of the dealkalized surface; the former is performed on powdered glass, which exposes internal surfaces of the glass compound. The results are based upon the amount of alkali titrated by 0.02 N sulfuric acid after an autoclaving cycle with the glass sample in contact with a high-purity distilled water. Thus, the *Powdered Glass Test* challenges the leaching potential of the interior structure of the glass while the *Water Attack Test* challenges only the intact surface of the container.

Selecting the appropriate glass composition is a critical facet of determining the overall specifications for each parenteral formulation.

In general, Type I glass will be suitable for all products, although sulfur dioxide treatment sometimes is used for a further increase in resistance. Because cost must be considered, one of the other less expensive types may be acceptable. Type II glass may be suitable, for example, for a solution which is buffered, has a pH below 7 or is not reactive with the glass. Type III glass usually will be suitable principally for anhydrous liquids or dry substances. However, some manufacturer-to-manufacturer variation in glass composition should be anticipated within each glass type. Therefore, for highly chemically sensitive parenteral formulations it may be necessary to specify both USP Type and a specific manufacturer.

Physical Characteristics—Some of the physical shapes of glass ampuls and vials are illustrated in Fig 3. Commerically available containers vary in size from 0.5 to 1000 mL. Sizes up to 100 mL may be obtained as ampuls and vials, and larger sizes as bottles. The latter are used mostly for intravenous and irrigating solutions. Smaller sizes are also available as cartridges. Ampuls and cartridges are drawn from glass tubing. The smaller vials may be made by mold-

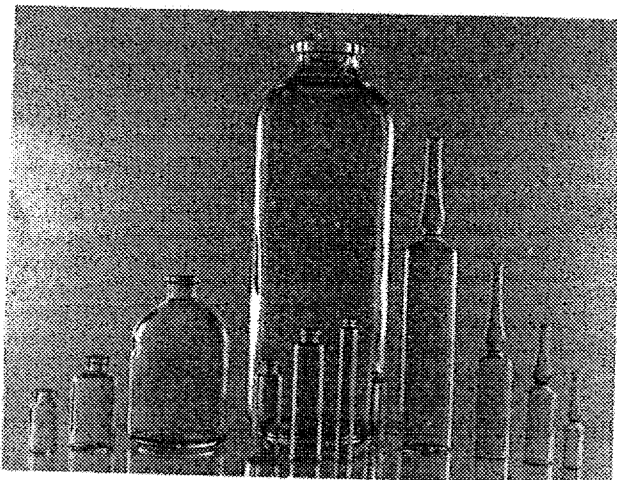


Fig 4. Various types of ampuls and multiple-dose vials for parenterals (courtesy, Kimble).

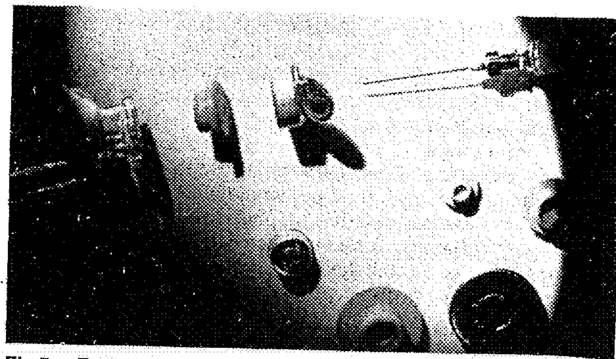


Fig 5. Extended view of scaling components for a multiple-dose vial (courtesy, West).

ing or from tubing. Larger vials and bottles are made only by molding. Containers made by drawing tubing are generally optically clearer and have a thinner wall than molded containers (see Fig 4). Molded containers are uniform in external dimensions, stronger and heavier.

Easy-opening ampuls that permit the user to break off the tip at the neck constriction without the use of a file are weakened at the neck by scoring or applying a ceramic paint having a different coefficient of thermal expansion. An example of a modification of container design to meet a particular need is the double-chambered vial, under the name Univial (*Univial*), designed to contain a freeze-dried product in the lower and solvent in the upper chamber. Other examples are wide-mouth ampuls with flat or rounded bottoms to facilitate filling with dry materials or suspensions, and various modifications of the cartridge for use with disposable dosage units.

Glass containers must be strong enough to withstand the physical shocks of handling and shipping and the pressure differentials that develop, particularly during the autoclave sterilization cycle. They must be able to withstand the thermal shock resulting from large temperature changes during processing, for example, when the hot bottle and contents are exposed to room air at the end of the sterilization cycle. Therefore, a glass having a low coefficient of thermal expansion is necessary. The container also must be transparent to permit inspection of the contents.

Preparations which are light-sensitive must be protected by placing them in amber glass containers or by enclosing flint glass containers in opaque cartons labeled to remain on the container during the period of use. It should be noted that the amber color of the glass is imparted by the incorporation of potentially leachable heavy metals, mostly iron and manganese, which may act as catalysts for oxidative degradation reactions. Silicone coatings sometimes are applied to containers to produce a hydrophobic surface, for example, as a means of reducing the friction of a rubber-tip of a syringe plunger.

The size of single-dose containers is limited to 1000 mL by the USP and multiple-dose containers to 30 mL, unless stated otherwise in a particular monograph. Multiple-dose vials are limited in size to reduce the number of punctures for withdrawing doses and the accompanying risk of contamination of the contents. As the name implies, single-dose containers are opened with aseptic care and the contents used at one time. These may range in size from 1000-mL bottles to 1-mL or less ampuls, vials or syringes. The integrity of the container is destroyed when opened so that the container cannot be closed again.

A multiple-dose container is designed so that more than one dose can be withdrawn at different times, the container maintaining a seal between uses. It should be evident that with full aseptic precautions, including sterile syringe and needle for withdrawing the dose and disinfection of the exposed surface of the closure, there is still a substantial risk of introducing contaminating microorganisms and viruses into the contents of the vial. Because of this risk, the USP requires that all multiple-dose vials must contain an antimicrobial agent or be inherently antimicrobial, as determined by the USP *Antimicrobial Preservatives-Effectiveness* tests. There are no comparable antiviral effectiveness tests, nor are antiviral agents available for such use. In spite of the advantagious flexibility of dosage provided by multiple-dose vials, single-dose, disposable container units provide the clear advantage of greater sterility assurance and patient safety.

Rubber Closures

In order to permit introduction of a needle from a hypodermic syringe into a multiple-dose vial and provide for resealing as soon as the needle is withdrawn, each vial is sealed with a rubber closure held in place by an aluminum cap. Figure 5 illustrates how this is done. This principle also is followed for single-dose containers of the cartridge type, except that there is only a single introduction of the needle to make possible the withdrawal or expulsion of the contents.

Rubber closures are composed of multiple ingredients that are plasticized and mixed together at an elevated temperature on milling machines. Subsequently, the plasticized mixture is placed in molds and vulcanized (cured) under high temperature and pressure. During vulcanization the polymer strands are cross-linked by the vulcanizing agent, assisted by the accelerator and activator, so that motion is restricted and the molded closure acquires the elastic, resilient character required for its use. Ingredients not involved in the cross-linking reactions remain dispersed within the compound and,

Table 2—Examples of Ingredients in Rubber Closures

Ingredient	Examples
Elastomer	Natural rubber (latex)
	Butyl rubber
	Neoprene
Vulcanizing (curing) agent	Sulfur
	Peroxides
Accelerator	Zinc dibutyldithiocarbamate
Activator	Zinc oxide
	Stearic acid
Antioxidant	Dilauryl thiodipropionate
Plasticizer/lubricant	Paraffinic oil
	Silicone oil
Fillers	Carbon black
	Clay
	Barium sulfate
Pigments	Inorganic oxides
	Carbon black

along with the degree of curing, affect the properties of the finished closure. Examples of rubber-closure ingredients are given in Table 2.

The physical properties to be considered in the selection of a particular formulation include elasticity, hardness, tendency to fragment and permeability to vapor transfer. The elasticity is critical in establishing a seal with the lip and neck of a vial or other opening, and in resealing after withdrawal of a hypodermic needle from a vial closure. The hardness should provide firmness by not excessive resistance to the insertion of a needle through the closure, while minimal fragmentation of pieces of rubber should occur as the hollow shaft of the needle is pushed through the closure. While vapor transfer occurs to some degree with all rubber formulations, appropriate selection of ingredients makes it possible to control the degree of permeability. Physicochemical and toxicological tests for evaluating rubber closures are described in section ⟨381⟩ in the USP.

The ingredients dispersed throughout the rubber compound may be subject to leaching into the product contacting the closure. These ingredients, examples of which are given in Table 2, pose potential compatibility interactions with product ingredients if leached into the product solution, and these effects must be evaluated.[14,15] Further, some ingredients must be evaluated for potential toxicity. In order to reduce the problem of leachables, attempts have been made to coat the product contact surfaces of closures with various polymers, the most successful being Teflon, or to treat the closures in ways considered trade secrets.

The physical shape of some typical closures may be seen in Fig 5. Most of them have a lip and a protruding flange that extends into the neck of the vial or bottle. Many disk closures are being used now, particularly in the high-speed packaging of antibiotics. Slotted closures are used on freeze-dried products to make it possible to insert the closure part way into the neck of the vial during the drying phase of the cycle. Partial insertion provides limited protection from contamination while permitting water vapor to escape from the drying product. The plunger type is used to seal one end of a cartridge. At the time of use, the plunger expels the product by a needle inserted through the closure at the distal end of the cartridge. Intravenous solution closures often have permanent holes for adapters of administration sets; irrigating solution closures usually are designed for pouring.

Production Facilities

A product having components of the best quality quickly may become totally unacceptable if the environment in which it is processed is contaminated or if the manufacturing procedure is not carried out properly. Therefore, the production facilities and the procedure used in processing the product must meet standards adequate for the task. The nearer these standards approach perfection, the better and safer should be the product.

Arrangement of Area

The production area can be considered in terms of five functional areas: the cleanup area, the compounding area, the aseptic area, the quarantine area and the finishing or packaging area. All of these should be designed and constructed for cleaning ease, appropriate environmental control, efficient operation and personnel comfort. The extra requirements for the aseptic area are designed to provide an environment where, for example, an injection may be exposed to the environment for a brief period during subdivision from a bulk container to the individual-dose containers without becoming contaminated. Contaminants such as dust, lint and microorganisms normally are found floating in the air, lying on counters and other surfaces, on clothing and body surfaces of personnel, in the exhaled breath of personnel and deposited on the floor. The design and control of an aseptic area is directed toward so reducing the presence of these contaminants that they are no longer a hazard to aseptic filling. Although the aseptic area must be adjacent to support areas so that an efficient flow of components may be achieved, barriers must be provided to minimize ingress of contaminants to the critical aseptic area. Such barriers may consist of a variety of forms, including sealed walls, manual or automatic doors, airlock pass-throughs, ports of various types or plastic curtains. Figure 6 shows an example of a floor plan in which the two fill rooms and the staging area constitute the walled critical aseptic area, access to which is only by means of pass-through airlocks. Adjacent support areas (rooms) consist of glass preparation, equipment wash, capping, manufacturing (compounding) and various storage areas. Figure 7 shows an adjacent arrangement with the utilization of a through-the-wall port for passage of a filtrate into the critical aseptic filling room.

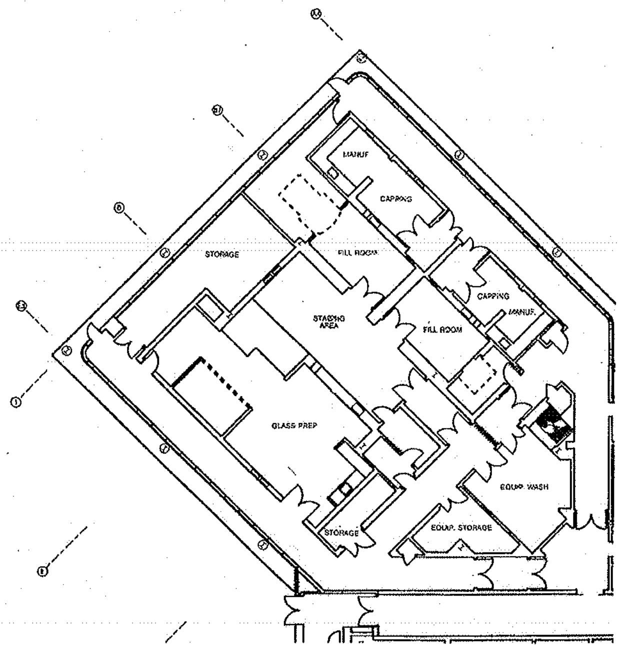


Fig 6. Floor plan of aseptic filling rooms and staging room with adjacent support areas (courtesy, Glaxo).

Flow Plan—In general, the components for a parenteral product flow from the stockroom for released components, either to the compounding area, as for ingredients of the formula, or to the cleanup area, as for containers and equipment. See Fig 8 for a process-flow diagram. After proper processing in these areas, the components flow into the security of the aseptic area for filling of the product in appropriate containers. From there the product passes into the quarantine area where it is held until all necessary tests have been performed. If the product is to be sterilized in its final container, its passage normally is interrupted after leaving the aseptic area for subjection to the sterilization process. After the results from all tests are known and the product has been found to comply with its release specifications, it passes to the finishing area for final labeling and packaging. There sometimes are variations from this flow plan to meet the specific needs of an individual product or to conform to existing facilities. Automated operations convey the components

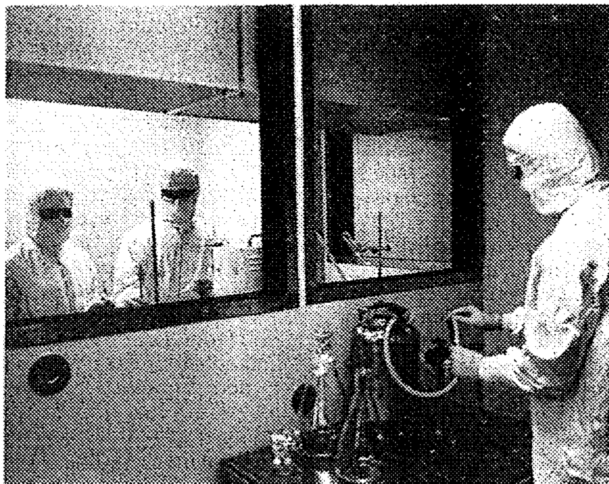


Fig 7. Product filtration from the aseptic staging room through a port into the aseptic filling room (courtesy, The University of Tennessee College of Pharmacy).

from one area to another with little or no handling by operators.

Cleanup Area—The cleanup area is constructed to withstand moisture, steam and detergents. The ceiling, walls and floor should be constructed of impervious materials so that moisture will run off and not be held. One of the "spray-on-tile" finishes with a vinyl or expoxy sealing coat provides a continuous surface free from all holes or crevices. All such surfaces can be washed at regular intervals to keep them thoroughly clean. These areas should be exhausted adequately so that the heat and humidity will be removed for the comfort of personnel. Precautions must be taken to prevent the accumulation of dirt and the growth of microorganisms, especially in the presence of high humidity and heat. In this area preparation for the filling operation, such as assembling equipment, is undertaken. Adequate sink and counter space must be provided. While this area does not need to be aseptic, it must be cleanable and kept clean and the microbial load must be monitored and controlled. Precautions also must be taken to prevent deposit of particles or other contaminants on clean containers and equipment.

Compounding Area—In this area the formula is compounded. Although it is not essential that this area be aseptic, control of microorganisms and particulates should be more stringent than in the cleanup area. For example, means may need to be provided to control dust generated from weighing and compounding operations. Cabinets and counters should, preferably, be constructed of stainless steel. They should fit snugly to walls and other furniture so that there are no catch areas for dirt to accumulate. The ceiling, walls and floor should be constructed similar to those for the cleanup area.

Aseptic Area

This area requires construction features designed for maximum microbial and particulate control. The ceiling, walls and floor must be sealed so that they may be washed and sanitized with a disinfectant, as needed. All counters should be constructed of stainless steel and hung from the wall so that there are no legs to accumulate dirt where they rest on the floor. All light fixtures, utility service lines and ventilation fixtures should be recessed in the walls or ceiling to eliminate ledges, joints and other locations for the accumulation of dust and dirt. As much as possible, tanks containing the compounded product should remain outside the aseptic filling area and the product fed into the area through hose lines. Figure 7 shows such an arrangement. Proper sanitization is required if the tanks must be moved in. Mechanical equipment that is located in the aseptic area should be housed as completely as possible within a stainless-steel cabinet in order to seal the operating parts and their dirt-producing tendencies from the aseptic environment. Mechanical parts that will contact the parenteral product should be demountable so that they can be sterilized.

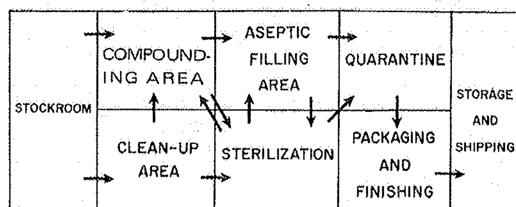


Fig 8. Process-flow diagram.

Personnel entering the aseptic area should enter only through an airlock. They should be attired in sterile coveralls with sterile hats, masks, goggles and foot covers. Movement within the room should be minimal and in-and-out movement rigidly restricted during a filling procedure. The requirements for room preparation and the personnel may be relaxed somewhat if the product is to be sterilized terminally in a sealed container. Some are convinced, however, that it is better to have one standard procedure meeting the most rigid requirements.

Air Cleaning

The air in these areas can be one of the greatest sources of contamination. It need not be, however, because several methods are available for providing clean air that is essentially free from dirt particles and microorganisms.

To provide such air, it must be cleaned thoroughly of all contaminants. This may be done by a series of treatments that will vary somewhat from one installation to another. One such series is air from the outside first is passed through a prefilter, usually of glass wool, cloth or shredded plastic, to remove large particles. Then it is treated by passage through an electrostatic precipitator (Suppliers: *Am Air, Electro-Air, Sturtevant*). Such a unit induces an electrical charge on particles in the air and removes them by attraction to oppositely charged plates. The air then passes through the most efficient cleaning device, a HEPA (high efficiency particulate air) filter having an efficiency of at least 99.97% in removing particles of 0.3 μm and larger, based on the DOP (Dioctyl phthalate) test (Suppliers: *Am Air, Cambridge, Flanders*).

For personnel comfort, air conditioning and humidity control should be incorporated into the system. Another system, the Kathabar system (*Surface Combustion*), cleans the air of dirt and microorganisms by washing it in an antiseptic solution and, at the same time, controls the humidity. The clean, aseptic air is introduced into the aseptic area and maintained under positive pressure, which prevents outside air from rushing into the aseptic area through cracks, temporarily open doors or other openings.

Laminar-Flow Environments—The required environmental control of aseptic areas has been made possible by the use of laminar airflow enclosures. Laminar airflow provides a total sweep of a confined space because the entire body of air moves with a uniform velocity, usually 90 ft/min, ± 20% along parallel lines, originating through a HEPA filter occupying one entire side of the confined space. Therefore, it bathes the total space with very clean air, sweeping away contaminants.

The arrangement for the direction of airflow can be horizontal (see Fig 9) or vertical (see Fig 10), and may involve a limited area such as a workbench or an entire room.

Figure 10 shows a vial-filling line protected with vertical laminar airflow from ceiling-hung HEPA filters. Plastic curtains are installed to maintain the laminarity of airflow to below the filling line and to circumscribe the critical filling portion of the line. The area outside the curtains can be

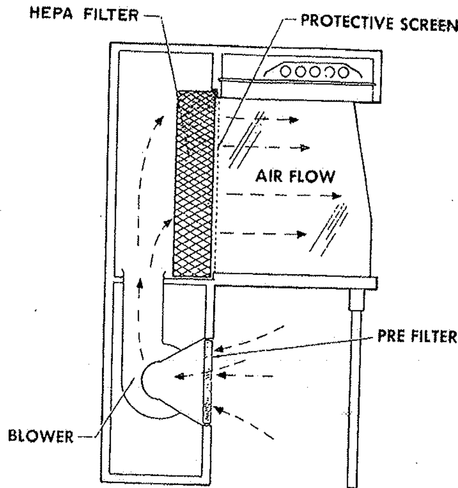


Fig 9. Horizontal laminar-flow workbench (courtesy, adaptation, Sandia).

maintained at a slightly lower level of cleanliness than that inside.

Laminar flow of HEPA-filtered air is capable of meeting the standard for a Class 100 clean room as defined by Federal Standard 209E,[16] which states that such an environment contains no more than 100 particles/ft^3 of 0.5 μm and larger in size. Thus, in Fig 10, the area within the curtains should be a Class 100 environment but the area outside may be Class 10,000 or cleaner, defined on the same basis. Today, it is accepted that critical areas of processing, wherein the product or product contact surfaces may be exposed to the environment, even for a brief period of time, should meet Class 100 clean room standards.

It must be borne in mind that any contamination introduced upstream by equipment, arms of the operator or leaks in the filter will be blown downstream. In the instance of horizontal flow this may be to the critical working site, the face of the operator or across the room. Should the contaminant be, for example, penicillin powder, a biohazard material or viable microorganisms, the danger to the operator is apparent. Further, great care must be exercised to prevent cross-contamination from one operation to another, especially with horizontal laminar air flow. For operations involving such contaminants a vertical system is much more desirable, with the air flowing through perforations in the countertop or through return louvers at floor level, where it can be directed for decontamination. Vertical flow has been recommended for sterility-testing procedures.

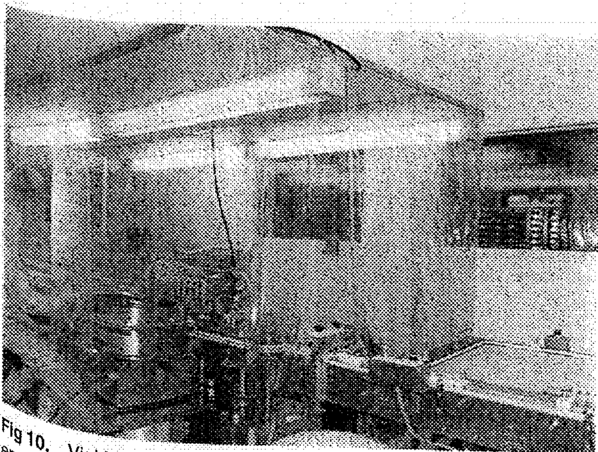


Fig 10. Vial filling line under vertical laminar airflow with critical area enclosed within plastic curtains (courtesy, Merck).

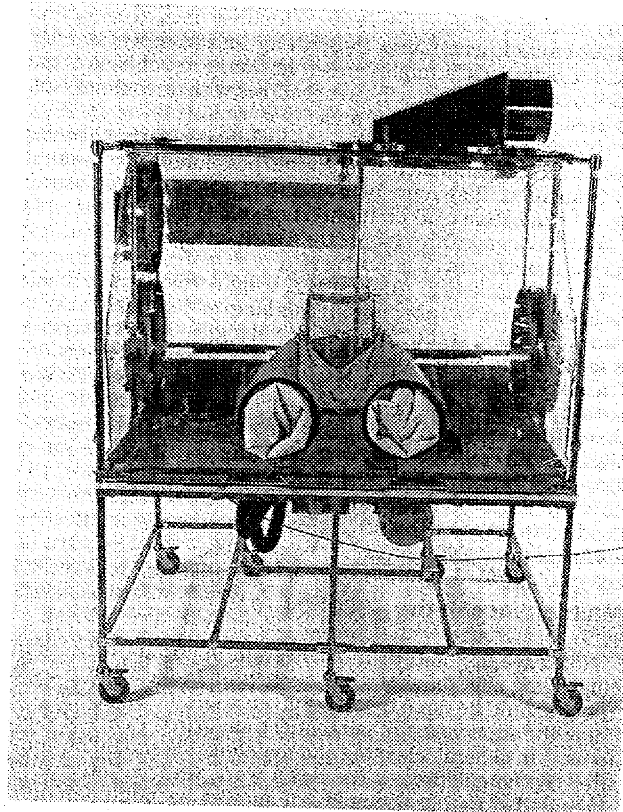


Fig 11. One configuration of an isolator (courtesy, Amsco).

Laminar-flow environments provide well-controlled work areas only if proper precautions are observed. Any reverse air currents or movements exceeding the velocity of the HEPA-filtered airflow may introduce contamination, as may coughing, reaching or other manipulations of operators.

Therefore, laminar-flow work areas should be protected by being located within controlled environments. Personnel should be attired for aseptic processing as described below. All movements and processes should be planned carefully to avoid the introduction of contamination upstream of the critical work area. Checks of the airstream should be performed initially and at regular intervals to be sure no leaks have developed through or around the HEPA filters.

Workbenches and other types of laminar-flow enclosures are available from several commercial sources (Suppliers: *Air Control, Atmos-Tech, Baker, Clean Air, Clestra, EACI/ Envirco, Flanders, Laminaire, Liberty, Veco*).

Isolation (barrier) technology is a relatively new approach designed to isolate aseptic operations from personnel. Figure 11 shows the structure of one configuration of an isolator. As can be seen, the operations are performed within transparent, plastic, sealed walls with the operator, in this instance, working through gloves and a half-suit. These isolation work stations are presterilized, usually with peracetic acid or hydrogen peroxide vapor, and sterile supplies are introduced from sterilizing modules through uniquely engineered transfer ports. Results from the use of these units in controlling contamination during sterility testing have reduced false positives to essentially zero. Studies are being undertaken to evaluate these units for aseptic production operations.[17]

Ultraviolet Radiation

Ultraviolet (UV) light rays have an antibacterial action, thereby producing a disinfectant action on directly irradiated surfaces. Since these rays cannot penetrate most materials,

only a surface effect is produced, with the principal exception being limited penetration through air and pure water. UV light rays travel in straight lines only; therefore, objects in the path of the light beam will cast shadows with a resultant lack of irradiation in the shadow area.

UV rays are irritating to the skin and, particularly, the eyes of human beings. Therefore, should personnel be in the area of irradiation, they must be protected from direct exposure. Direct irradiation of a room when personnel are not present is a valuable means of reducing the bacterial count on working surfaces and floors.

The best practical source of UV light rays is the cold-cathode mercury vapor lamp. This lamp emits a high proportion of radiation at the 253.7 nm wavelength. A special glass is used for the tube so that the rays will pass to the outside. This glass gradually will change in crystal structure with use so that passage of the rays is gradually reduced. Such lamps, therefore, rarely burn out as do visible-light lamps but gradually reach an emission level which is ineffective. These lamps also must be kept clean, for dust and grease will lower the effective emission drastically. It generally is stated that an irradiation intensity of 20 $\mu w/cm^2$ is required for effective antibacterial activity.

Maintenance of the Aseptic Area

Housekeeping and maintenance are important aspects in controlling environmental contamination in the aseptic area. These should be done by crews given special instruction and under the supervision of personnel trained in the care of such areas. In general, cleaning and maintenance should be done after the completion of the day's work with an interval of quietude before the beginning of another aseptic operation. With the advent of laminar flow of HEPA-filtered air the rigors of cleaning have been reduced since the clean airflow continuously "sweeps" the area clean. All maintenance equipment should be selected for its effectiveness and freedom from lint-producing tendencies and should be reserved for use in aseptic areas only.

Personnel

Personnel selected to work on the preparation of a parenteral product must be neat, orderly and reliable. They should be in good health and free from dermatological conditions that might increase the microbial load. If they show symptoms of a head cold, allergies or similar illness, they should not be permitted in the aseptic area until their recovery is complete. However, a healthy person with the best personal hygiene still will shed large numbers of viable and nonviable particles from body surfaces. This natural phenomenon creates continuing problems when personnel are present in clean rooms, but effective training and proper gowning can reduce, but not eliminate, the problem of particle shedding from personnel.

Aseptic-area operators should be given thorough, formal training in the principles of aseptic processing and the techniques to be employed. Subsequently, the acquired knowledge and skills should be evaluated, to be sure training has been effective, before they are allowed to participate in the preparation of sterile products. Retraining should be performed on a regular schedule to enhance the maintenance of the required level of expertise. An effort should be made to imbue operators with an awareness of the vital role they play in determining the reliability and safety of the final product. This is especially true of supervisors since they should be individuals who not only understand the unique requirements of aseptic procedures but who are able to obtain the full participation of other employees in fulfilling these exacting requirements.

The uniform worn is designed to confine the contaminants discharged from the body of the operator, thereby preventing their entry into the production environment. For use in the aseptic area, uniforms should be sterile. Fresh, sterile uniforms should be used after every break period, or whenever the individual returns to the aseptic area. In some plants this is not required if the product is to be sterilized in its final container. The uniform usually consists of coveralls for both men and women, hoods to completely cover the hair, face masks and Dacron or plastic boots (Fig 12). Sterile rubber gloves also are required for aseptic operations, preceded by thorough scrubbing of the hands with a disinfectant soap. In addition, goggles may be required to complete the coverage of all skin areas.

Dacron or Tyvek uniforms are used usually, are effective barriers to discharged body particles (viable and nonviable), are essentially lint-free and are reasonably comfortable. Air showers are sometimes directed on personnel entering the processing area to blow loose lint from the uniforms.

Environmental Control Evaluation

As evidenced by the above discussion, manufacturers of sterile products use extensive means to control the environment so that these critical products can be prepared free from contamination. Nevertheless, tests should be performed to determine the level of control actually achieved. Normally, the tests consist of counting viable and nonviable particles suspended in the air or settled on surfaces in the workspace. A baseline count, determined by averaging multiple counts when the facility is operating under controlled conditions, is used to establish the optimal test results expected. During the subsequent monitoring program, the test results are followed carefully for high individual counts, a rising trend or other abnormalities. If they exceed selected alert or action levels, a plan of action must be put into operation to determine if or what corrective measures are required.

The tests used generally measure either the particles in a volume of sampled air or the particles that are settling or are present on surfaces. A volume of air measured by an elec-

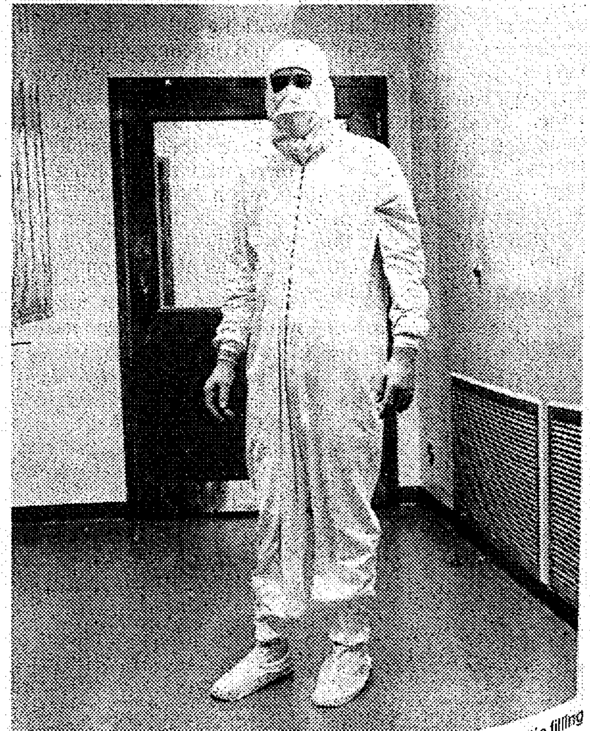


Fig 12. Appropriate uniform for operators entering an aseptic filling room (courtesy, Abbott).

tronic particle counter will detect all particles instantly, but not differentiate between viable and nonviable ones. However, because of the need to control the level of microorganisms in the environment in which sterile products are processed, it also is necessary to detect viable particles. These usually are less in number than nonviable ones and are only detectable as colony forming units (CFUs) after a suitable incubation period at, for example, 30 to 35°C for up to 48 hours.

Locations for sampling should be planned to reveal potential contamination levels which may be critical in the control of the environment. For example, the most critical process step is usually the filling of dispensing containers, a site obviously requiring monitoring. Other examples include the gowning room, high-traffic sites in and out of the filling area, the penetration of conveyor lines through walls and sites near the inlet and exit of the air system.

The size of the sample should be large enough to obtain a meaningful particle count. At sites where the count is expected to be low the size of the sample may need to be increased; for example, in Class 100 areas, Whyte and Niven,[18] suggest that the sample should be at least 30 ft^3 and, probably, much more. They also suggest that settling plates should be exposed in Class 100 areas for an entire fill (up to 7 to 8 hours) rather than the more common 1 hour. However, excessive dehydration of the medium must be avoided, particularly in the path of laminar-flow air.

To measure the total particle content in an air sample, electronic particle counters are available, operating on the principle of the measurement of light-scattered from particles as they pass through the cell of the optical system (Suppliers: *Climet, Met One, Particle Measuring, Royco*). These instruments not only count particles but also provide a size distribution based on the magnitude of the light scattered from the particle.

Several air-sampling devices are used to obtain a count of microorganisms in a measured volume of air. A slit-to-agar (STA) sampler (Suppliers: *Mattson-Garvin, New Brunswick*) draws by vacuum a measured volume of air through a narrow opening causing the air to impact on the surface of a slowly rotating nutrient agar plate. Microorganisms adhere to the surface of the agar and grow into visible colonies which are counted as CFUs, since it is not known whether the colonies arise from a single microorganism or a cluster. A centrifugal sampler (Supplier: *Biotest*) pulls air into the sampler by means of a rotating propeller and slings the air by centrifugal action against a peripheral nutrient agar strip. The advantages of this unit are that it can be disinfected easily and is portable so that it can be hand-carried wherever needed. These two methods are used quite widely.

A widely used method for microbiological sampling consists of the exposure of nutrient agar culture plates to the settling of microorganisms from the air. This method is very simple and inexpensive to perform but will detect only those organisms which have settled on the plate; therefore, it does not measure the number of microorganisms in a measured volume of air. Nevertheless, if the conditions of exposure are repeated consistently, a comparison of CFUs at one sampling site from one time to another can be meaningful.

The number of microorganisms on surfaces can be determined with nutrient agar plates having a convex surface (*Rodac Plates*). With these it is possible to roll the raised agar surface over flat or irregular surfaces to be tested. Organisms will be picked up on the agar and will grow during subsequent incubation. This method also can be used to assess the number of microorganisms present on the surface of the uniforms of operators, either as an evaluation of gowning technique immediately after gowning or as a measure of the accumulation of microorganisms during processing. Whenever used, care must be taken to remove any agar residue left on the surface tested.

Further discussion of proposed viable particle test methods and the counts to be accepted will be found in Section ⟨1116⟩ "Microbial Evaluation and Classification of Clean Rooms and Clean Zones" in *Pharm Forum 18:*4048, 1992.

Results from the above tests are very valuable to keep cleaning, production and quality-control personnel apprised of the level of contamination in a given area and, by comparison with baseline counts, will indicate when more extensive cleaning and sanitizing is needed. The results also may serve to detect environmental control defects such as failure in air-cleaning equipment or the presence of personnel who may be disseminating large numbers of bacteria without apparent physical ill effects.

Media Fill—An evaluation which is not strictly an environmental test, but which includes an evaluation of the environment along with the process, the operators and the equipment, is the "media fill." Sterile trypticase soy broth is filled into sterile containers under conditions simulating as closely as possible those characteristics of a filling process for a product. The entire lot, normally at least 3000 units, is then incubated at a suitable temperature, usually 20 to 25°, for at least 14 days and examined for the appearance of growth of microorganisms. If growth occurs, contamination has entered the container(s) during the processing. To pass the test not more than 0.1% of the units may show growth. This evaluation also has been used as a measure of the proficiency of an individual operator. This test is a very stringent evaluation of the efficiency of an aseptic filling process and, by many, is considered to be the most evaluative test available.

Production Procedures

The processes required for preparing sterile products constitute a series of events initiated with the securing of approved raw materials (drugs, excipients, vehicles, etc) and primary packaging components (containers, closures, etc) and ends with the sterile product sealed in its dispensing package. Each step in the process must be controlled very carefully in order that the product will have its required quality. To assure the latter, each process should be validated to be sure that it is accomplishing what it is intended to do. For example, an autoclave sterilization process must be validated by providing data showing that it effectively kills resistant forms of microorganisms; or, a cleaning process for rubber closures should provide evidence that it is cleaning closures to the required level of cleanliness. The validation of processes requires an extensive and intensive effort to be successful, and is an integral part of CGMP requirements.

In the following sections the production procedures used in preparing sterile drug products will be discussed.

Cleaning Containers and Equipment

Containers and equipment coming in contact with parenteral preparations must be cleaned meticulously. It is obvious that if this were not so, all other precautions to prevent contamination of the product would be useless. It also should be obvious that even new, unused containers and equipment will be contaminated with such debris as dust, fibers, chemical films and other materials arising from such sources as the atmosphere, cartons, the manufacturing process and human hands. Much greater contamination must be removed from previously used equipment before it will be suitable for reuse. Equipment should be reserved exclusively for use only with

parenteral preparations and, where conditions dictate, only for one product in order to reduce the risk of contamination.

A variety of machines are available for cleaning containers for parenteral products. These vary in complexity from a small rotary rinser (Fig 13) to large automatic washers capable of processing several thousand containers an hour (Fig 14). The selection of the particular type will be determined largely by the physical type of containers, their condition with respect to contamination and the number to be processed in a given period of time.

Characteristics of Machinery—Regardless of the type of cleaning machine selected, certain fundamental characteristics usually are required.

1. The liquid or air treatment must be introduced in such a manner that it will strike the bottom of the inside of the inverted container, spread in all directions and smoothly flow down the walls and out the opening with a sweeping action. The pressure of the jet stream should be such that there is minimal splashing, and the flow should be such that it can leave the container opening without accumulating and producing turbulence inside. Splashing may prevent cleaning all areas, and turbulence may redeposit loosened debris. Therefore, direct introduction of the jet stream within the container with control of its flow is required.
2. The container must receive a concurrent outside rinse.
3. The cycle of treatment should provide for a planned sequence alternating very hot and cool treatments. The final treatment should be an effective rinse with water of a quality equivalent to WFI.
4. All metal parts coming in contact with the containers and with the treatments should be constructed of stainless steel or some other noncorroding and noncontaminating material.

Treatment Cycle—The cycle of treatments to be employed will vary with the condition of the containers to be cleaned. In general, loose dirt can be removed by vigorous rinsing with water. Detergents rarely are used for new containers because of the risk of leaving detergent residues. However, a thermal-shock sequence in the cycle usually is employed to aid, by expansion and contraction, loosening of debris that may be adhering to the container wall. Sometimes only an air rinse is used for new containers, particularly if used for a dry powder. In all instances the final rinse, whether air or WFI, must be ultraclean so that no particulate residues are left by the rinsing agent.

Only new containers are used for parenterals. Improvements have been made in maintaining their cleanliness during shipment from the manufacturer through tight, low-shedding packaging, including plastic blister packs, as can be seen stacked on the right of Fig 14.

Machinery for Containers—The machinery available for cleaning containers embodies the above principles but varies in the mechanics by which it is accomplished. In one approach, the jet tubes are arranged on arms like the spokes of a wheel, which rotate around a center post through which the treatments are introduced. An operator places the unclean containers on the jet tubes as they pass the loading point and removes the clean containers as they complete one rotation. Such a small-scale machine is pictured in Fig 14. A washer capable of cleaning hundreds of containers an hour, shown in Figure 14, uses a row of jet tubes across a conveyor belt. The belt moves the inverted containers past the programmed series of treatments and discharges the clean containers into a sterilizing oven (not shown), which ultimately discharges them through a wall into a clean room for filling. Another type of machine is the rack-loading washer. Stainless-steel racks are designed to fit over the open ends of ampuls or vials as configured in trays of shipping cartons or blister packs. Inverting the trays permits the containers to slide into the racks so that they can be handled by the quantity in the tray, as shown in Fig 15. The clean containers may be transferred directly to the conveyor of a sterilizating tunnel (as shown), or they may be placed in stainless-steel boxes for subsequent dry-heat sterilization and storage. A continuous automated line operation is shown in Figure 16. The vials are fed into the rotary rinser in the foreground, transferred automatically to the covered sterilizing tunnel in the center, conveyed through the wall in the background and discharged through the wall into the filling clean room.

Handling after Cleaning—The wet, clean containers must

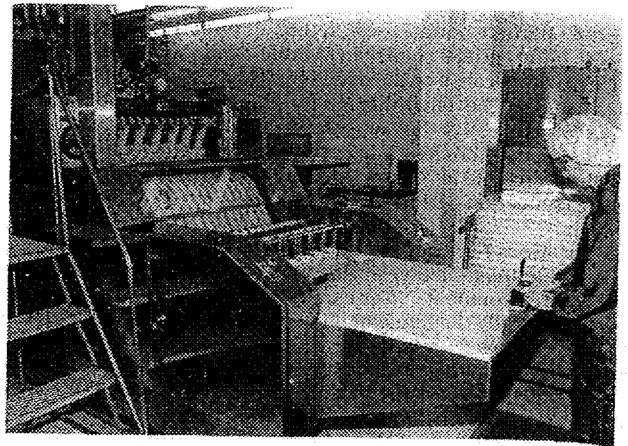


Fig 14. Loading end of large conveyor vial washer that subjects inverted vials to a series of cleaning steps before delivery from the far end of the washer. Note the vials in plastic blister packs at right of operator (courtesy, Merck).

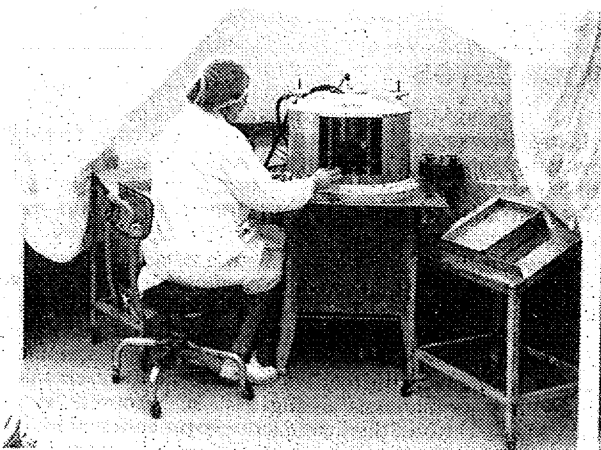


Fig 13. Rotary rinser (Cozzoli) in a clean environment provided by vertical laminar airflow within a curtained enclosure (courtesy, Ciba-Geigy).

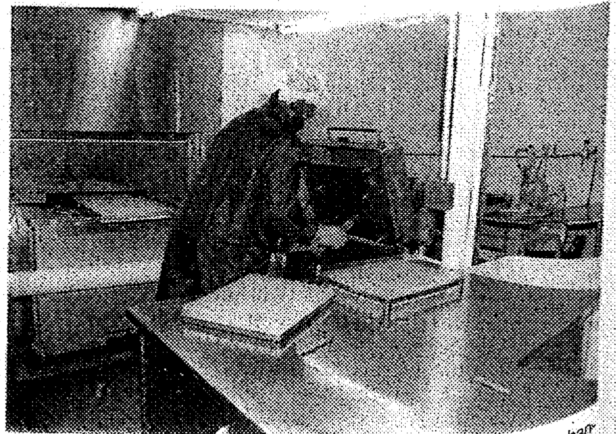
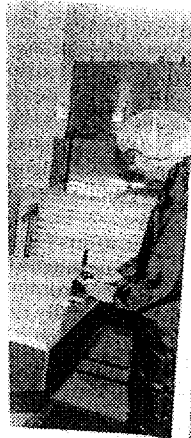


Fig 15. Cleaning vials with rack-loading washer, permitting handling vials by a full rack. After multiple-washing treatments, the racks are placed directly on the conveyor belt of the hot-air sterilizing tunnel (courtesy, Merck).



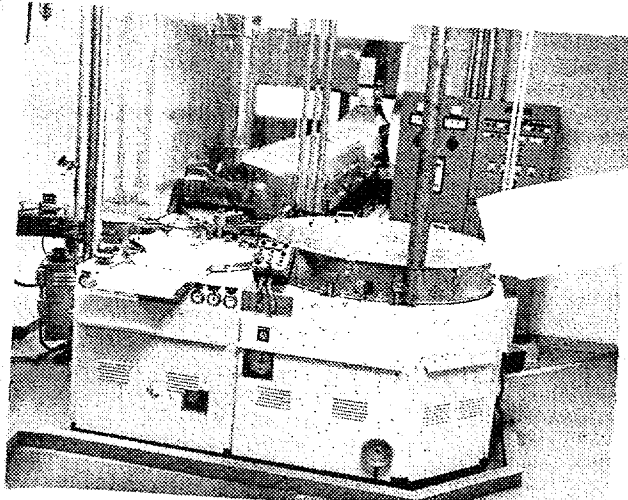


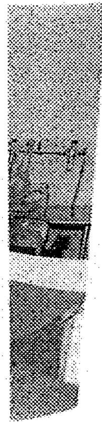
Fig 16. Continuous automatic line operation for vials from a rotary rinser through a sterilizing tunnel with vertical laminar-airflow protection of clean vials (courtesy, Abbott).

be handled in such a way that contamination will not be reintroduced. A wet surface will collect contaminants much more readily than will a dry surface. For this reason wet, rinsed containers must be protected, such as by a laminar flow of clean air until covered, as within a stainless-steel box or within a sterilizing tunnel. Although not clearly visible in each instance, the wet, clean containers in Figs 13–16 were so protected. In addition, microorganisms are more likely to grow in the presence of moisture. Therefore, wet, clean containers should be dry-heat sterilized as soon as possible after washing. Doubling the heating period generally has been considered to be adequate also to destroy pyrogens, but the actual time-temperature conditions required must be validated.

Increases in process rates have necessitated the development of continuous, automated line processing with a minimum of individual handling, still maintaining adequate control of the cleaning and handling of the containers. In Fig 16, the clean, wet containers are protected by filtered laminar-flow air from the rinser through the tunnel and until they are delivered to the filling line.

Closures—Rubber closures are coated with lubricant from the molding operation. In addition, the rough surface and electrostatic attraction tend to hold debris. Also, the surface "bloom" from migrated inorganic constituents of the compound must be removed. The recommended procedure calls for gentle agitation in a hot solution of a mild water softener. The closures are removed from the solution and rinsed several times, or continuously for a prolonged period, with filtered WFI. The rinsing is to be done in a manner which will flush away loosened debris. The wet closures are carefully protected from pick up of environmental contamination, are sterilized, usually by autoclaving, and stored in closed containers until ready for use. This cleaning and sterilizing process must be validated with respect to rendering the closures free from pyrogens. Actually, it is the cleaning and final, thorough rinsing with WFI that must remove pyrogens, since autoclaving does not destroy them. If the closures were immersed during autoclaving, the solution is drained off before storage to reduce hydration of the rubber compound. If the closures must be dry for use, they may be subjected to vacuum drying at a temperature in the vicinity of 100°.

The equipment used for washing large numbers of closures is usually an agitator or horizontal basket-type automatic washing machine. Because of particulate generation from the abrading action of these machines, some heat the closures in kettles in detergent solution and follow with prolonged flush rinsing. The final rinse always should be with low particulate WFI.



Equipment—The details of certain prescribed techniques for cleaning and preparing equipment, as well as of containers and closures, have been presented elsewhere.[19] Here, a few points will be emphasized.

All equipment should be disassembled as much as possible to provide access to internal structures. For thorough cleaning, surfaces should be scrubbed thoroughly with a stiff brush using an effective detergent, paying particular attention to joints, crevices, screw threads and other structures where debris is apt to collect. Exposure to a stream of clean steam will aid in dislodging residues from the walls of stationary tanks, spigots, pipes and similar structures. Thorough rinsing with distilled water should follow the cleaning steps. Large stationary tanks, such as those shown in Fig 17, should be protected as much as possible from contamination after cleaning but should be rinsed thoroughly again with distilled water or WFI prior to reuse.

Because of the inherent variation in the manual cleaning of tanks and other large equipment items, together with the need to validate the process, an automated, usually computer-controlled, system has been developed called "cleaning in place" (CIP).[20] Such an approach involves designing the system, normally of stainless steel, with smooth, rounded internal surfaces and without crevices. That is, for example, with welded rather than threaded connections. The cleaning is accomplished with the scrubbing action of high-pressure spray balls or nozzles delivering hot detergent solution from tanks captive to the system. Thorough rinsing with WFI follows and is accomplished within the same system. The system often is extended to permit sterilizing in place (SIP) as well.[21]

Rubber tubing, rubber gaskets and other rubber parts may be washed in a manner such as described for rubber closures. Thorough rinsing of tubing must be done by passing WFI through it. However, due to the relatively porous nature of rubber compounds and the difficulty in removing all traces of chemicals from previous use, it is considered by some inadvisable to reuse rubber tubing. Rubber tubing must be left wet when preparing for sterilization by autoclaving.

Product Preparation

The basic principles employed in the compounding of the product do not vary from those used routinely by qualified pharmacists. However, selected aspects will be mentioned for emphasis.

A master formula would have been developed and on file. Each batch formula sheet should be prepared from the master and confirmed for accuracy. All measurements of quantities

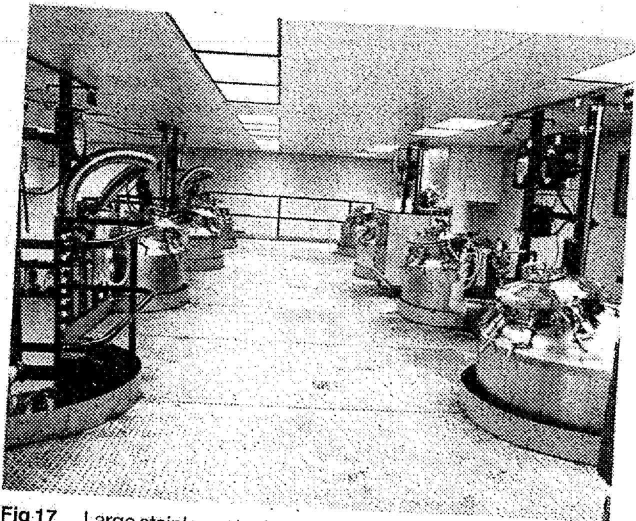


Fig 17. Large stainless-steel tanks for product preparation showing mezzanine access level (courtesy, Abbott).

should be made as accurately as possible and checked by a second qualified person. Frequently, today, the formula documents are generated by a computer and the measurements of quantities of ingredients computer controlled. Although most liquid preparations are dispensed by volume, they are prepared by weight, since weighings can be performed more accurately than volume measurements and no consideration needs to be given to the temperature.

Care must be taken that equipment is not wet enough to significantly dilute the product or, in the case of anhydrous products, to cause a physical incompatibility. The order of mixing of ingredients may affect the product significantly, particularly those of large volume where attaining homogeneity requires considerable mixing time. For example, the adjustment of pH by the addition of an acid, even though diluted, may cause excessive local reduction in the pH of the product so that adverse effects are produced before the acid can be dispersed throughout the entire volume of product.

Parenteral dispersions, including colloids, emulsions and suspensions, provide particular problems. Parenteral emulsions have been reviewed by Singh and Ravin.[22] In addition to the problems of achieving and maintaining proper reduction in particle size under aseptic conditions, the dispersion must be kept in a uniform state of suspension throughout the preparative, transfer and subdividing operations.

The formulation of a stable product is of paramount importance. Certain aspects of this have been mentioned in the discussion of components of the product. Exhaustive coverage of the topic is not possible within the limits of this text, but further coverage is provided in Chapter 83. It should be mentioned here, however, the thermal sterilization of parenteral products increases the possibility of chemical reactions. Such reactions may progress to completion during the period of elevated temperature in the autoclave, or be initiated at this time but continue during subsequent storage. The assurance of attaining product stability requires a high order of pharmaceutical knowledge and responsibility.

Filtration

After a product has been compounded, it must be filtered if it is a solution. The primary objective of filtration is to clarify a solution. A high degree of clarification is termed "polishing" a solution. This term is used when particulate matter down to approximately 2 μm in size is removed. A further step, removing particulate matter down to 0.2 μm in size, would eliminate microorganisms and would accomplish "cold" sterilization. A solution having a high degree of clarity conveys the impression of high quality and purity, desirable characteristics for a parenteral solution.

Filters are thought to function by one or, usually, a combination of the following: (1) sieving or screening, (2) entrapment or impaction and (3) electrostatic attraction. When a filter retains particles by sieving, they are retained on the surface of the filter. Entrapment occurs when a particle, smaller than the dimensions of the passageway (pore), becomes lodged in a turn or impacted on the surface of the passageway. Electrostatic attraction causes particles opposite in charge to that of the surface of the filter pore to be held or adsorbed to the surface. It should be noted that increasing, prolonging or varying the force behind the solution may tend to sweep particles initially held by entrapment or electrostatic charge through the pores and into the filtrate.

Membrane filters are used for parenteral solutions because of their particle-retention effectiveness, nonshedding property, nonreactivity and disposable characteristics. However, it should be noted that nonreactivity does not apply in all cases. For example, polypeptide products may show considerable adsorption through some membrane filters, but those composed of polysulfone and polyvinylidine difluoride have been developed to be essentially nonadsorptive for these products. The most common membranes are composed of

Cellulose ester (Suppliers: *Gelman, Millipore, Sartorius, Schleicher Seitz*).
Nylon (Supplier: *Pall*).
Polysulfone (Supplier: *Gelman*).
Polycarbonate (Supplier: *Nuclepore*).
Polyvinylidene difluoride (Supplier: *Millipore*).
Polytetrafluoroethylene (Teflon) (Supplier: *Millipore*).

They are available as flat membranes or pleated into cylinders to increase surface area and, thus, flow rate. Each filter in its holder should be tested for integrity before and after use, particularly if it is being used to eliminate microorganisms. This integrity test usually is performed as the *bubble-point test*, a test to detect the largest pore, or other opening, through the membrane. The basic test is performed by gradually raising air pressure on the upstream side of a water-wet filter. The pressure at which bubbles first appear downstream is the bubble point. This pressure is characteristic for each pore size of a filter and is provided by the filter manufacturer. For example, a 0.2 μm cellulose ester filter will bubble at about 50 psig. If the filter is wetted with other liquids, such as a product, the bubble point will differ and must be determined experimentally. If the bubble point is lower than the rated pressure, the filter is defective, probably due to a puncture or tear, and should not be used. As the surface area of filters becomes large, diffusion of air through the water-filled pores tends to obscure the bubble point. Therefore, a diffusion, or pressure hold, test has been developed as an integrity test for filters with large surface areas. Particulars are obtainable from the filter manufacturer.

While membrane filters are disposable, and thus discarded after use, the holders must be cleaned thoroughly between uses. Today, clean, sterile, pretested, disposable assemblies for small as well as large volumes of solutions are available commercially. Other characteristics of these filters, important for a full understanding of their use, are given in Chapter 84 and in a review article.[23]

Filling

During the filling of containers with a product, the most stringent requirements must be exercised to prevent contamination, particularly if the product has been sterilized by filtration and will not be sterilized in the final container. Under the latter conditions the process usually is called an "aseptic fill" and is validated with media fills (see page 1537). During the filling operation, the product must be transferred from a bulk container and subdivided into dose containers. This operation exposes the sterile product to the environment, equipment and manipulative technique of the operator until it can be sealed in the dose container. Therefore, this operation is carried out with a minimum exposure time in the aseptic filling area where maximum protection is provided. Additional protection may be provided by filling under a blanket of HEPA-filtered laminar-flow air within the aseptic area.

Normally, the compounded product is in the form of either a liquid or a solid. A liquid is more readily subdivided uniformly and introduced into a container having a narrow mouth than is a solid. Mobile, nonsticking liquids are considerably easier to transfer and subdivide than viscous, sticky liquids, which require heavy-duty machinery for rapid production filling.

Although many devices are available for filling containers with liquids, certain characteristics are fundamental to them all. A means is provided for repetitively forcing a measured volume of the liquid through the orifice of a delivery tube which is introduced into the container. The size of the delivery tube will vary from that of about a 20-gauge hypodermic needle to a tube ½ in. or more in diameter. The size required is determined by the physical characteristics of the liquid, the desired delivery speed and the inside diameter of the neck of the container. The tube must enter the neck and deliver the liquid well into the neck to eliminate spillage, allowing sufficient clearance to permit air to leave the container as the liquid enters. The delivery tube should be as large in diameter as possible in order to reduce the resistance to the flow of the liquid. For smaller volumes of liquids, the delivery usu-

ally is obtained from the stroke of the plunger of a syringe, forcing the liquid through a two-way valve providing for alternate filling of the syringe and delivery of mobile liquids. A sliding piston valve would be used for heavy, viscous liquids. Other mechanisms include the turn of an auger in the neck of a funnel or the oscillation of a rubber diaphragm. For large volumes the quantity delivered usually is measured in the container by the level of fill in the container, the force required to transfer the liquid being provided by gravity, a pressure pump or a vacuum pump.

The narrow neck of an ampul limits the clearance possible between the delivery tube and the inside of the neck. Since a drop of liquid normally hangs at the tip of the delivery tube after a delivery, the neck of an ampul will be wet as the delivery tube is withdrawn, unless the drop is retracted. Therefore, filling machines should have a mechanism by which this drop can be drawn back into the lumen of the tube.

Since the liquid will be in intimate contact with the parts of the machine through which it flows, these must be constructed of nonreactive materials such as borosilicate glass or stainless steel. In addition, they should easily be demountable for cleaning and sterilization.

Because of the concern for particulate matter in injectable preparations, a final filter often is inserted in the system between the filler and the delivery tube, as shown in Fig 18. Most frequently this is a membrane filter, having a porosity of approximately 1 μm and treated to have a hydrophobic edge. This is necessary to reduce the risk of rupture of the membrane due to filling pulsations. It should be noted that the insertion of the filter at this point should collect all particulate matter generated during the process. Only that which may be found in inadequately cleaned containers or picked up from exposure to the environment after passage through the final filter potentially remain as contaminants. However, the filter does cushion liquid flow and reduces the efficiency of drop retraction from the end of the delivery tube, sometimes making it difficult to control delivery volume as precisely as would be possible without the filter.

Liquids—The filling of a small number of containers may be accomplished with a hypodermic syringe and needle, the liquid being drawn into the syringe and forced through the needle into the container. A device for providing greater speed of filling is the Cornwall Pipet (*Becton Dickinson*). This has a two-way valve between the syringe and the needle and a means for setting the stroke of the syringe so that the same volume will be delivered each time. Clean, sterile, disposable assemblies (Suppliers: *Burron, Pharmaseal*) operating on the same principle have particular usefulness in hospital pharmacy operations.

Mechanically operated instruments substitute a motor for the operator's hand in the previous devices described. Thereby, a much faster filling rate can be achieved. By careful engineering, the stroke of the syringe can be repeated precisely, and so, once a particular setting has been calibrated to the delivery, high delivery precision is possible. However, the speed of delivery, the expansion of the rubber tubing connecting the valve with the delivery tube and the rapidity of action of the valves can affect the precision of delivery. A filling machine employing a piston valve is shown in Fig 18. Stainless-steel syringes are required with viscous liquids because glass syringes are not strong enough to withstand the high pressures developed during delivery.

When high-speed filling rates are desired but accuracy and precision must be maintained, multiple filling units often are joined together in an electronically coordinated machine, such as shown in Fig 19. When the product is sensitive to metals, a peristaltic-pump filler may be used because the product comes in contact only with silicone rubber tubing. However, there is some sacrifice of filling accuracy.

Most high-speed fillers for large-volume solutions use the bottle as the measuring device, transferring the liquid either by vacuum or positive pressure from the bulk reservoir to the individual unit containers. Therefore, a high accuracy of fill is not achievable.

To insure delivery of the labeled volume to the patient, the USP provides a table of suggested fill volumes to include a slight excess over labeled volume.

Solids—Sterile solids, such as antibiotics, are more difficult to subdivide evenly into containers than are liquids. The rate of flow of solid material is slow and irregular. Even though a container with a larger diameter opening is used to facilitate filling, it is difficult to introduce the solid particles, and the risk of spillage is ever-present. The accuracy of the quantity delivered cannot be controlled as well as with liquids. Because of these factors, the tolerances permitted for the content of such containers must be relatively large. Suggested tolerances can be found in the USP.

Some sterile solids are subdivided into containers by individual weighing. A scoop usually is provided to aid in approximating the quantity required, but the quantity filled into the container finally is weighed on a balance. This is a slow process. When the solid is obtainable in a granular form so that it will flow more freely, other methods of filling may be employed. In general, these involve the measurement and delivery of a volume of the granular material which has been calibrated in terms of the weight desired. In the machine shown in Fig 20 an adjustable cavity in the rim of a wheel is

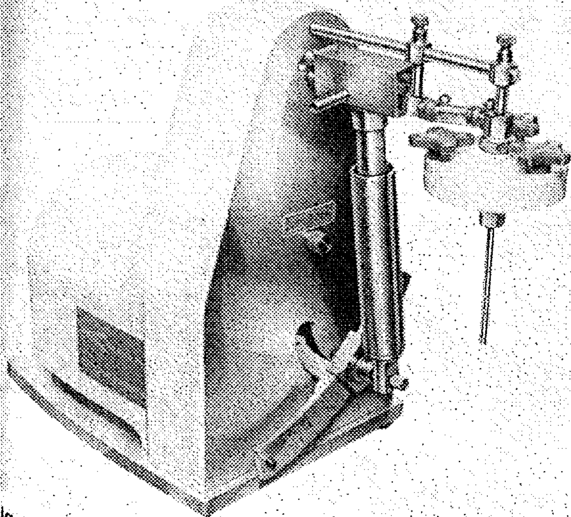


Fig 18. Filling machine employing a piston valve and a stainless-steel syringe (courtesy, Cozzoli).

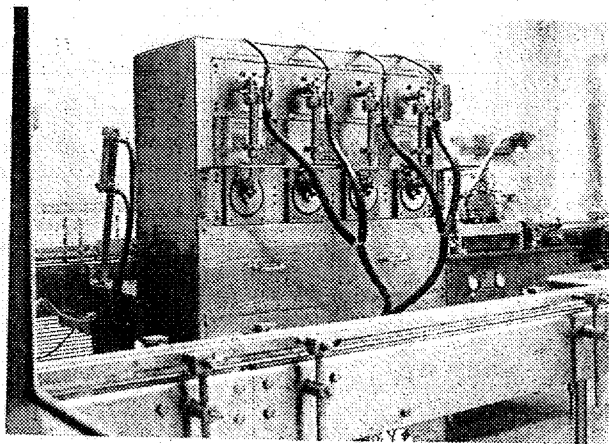


Fig 19. Four-pump liquid filler, with a conveyor line for vials protected by a vertical laminar airflow and plastic curtain; note the automatic stoppering machine on the right within the curtain (courtesy, Abbott).

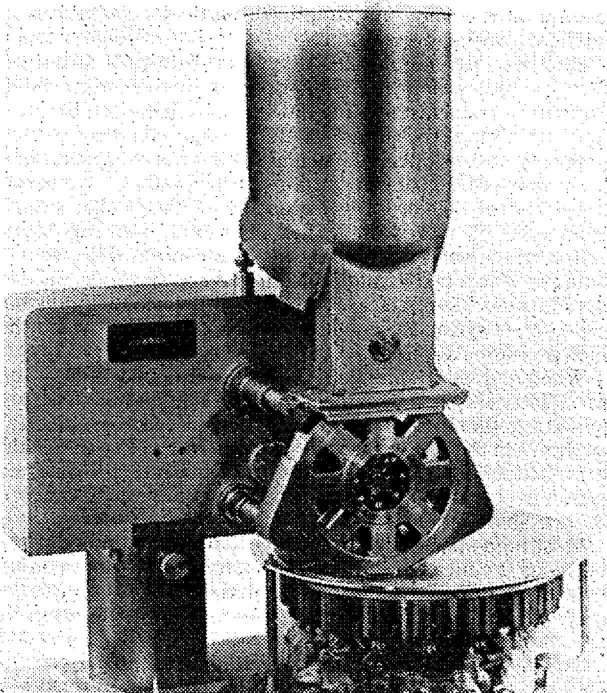


Fig 20. Accofil vacuum powder filler (courtesy, Perry).

filled by vacuum and the contents held by vacuum until the cavity is inverted over the container. The solid material then is discharged into the container by the use of sterile air. Another machine employs an auger in the stem of a funnel at the bottom of a hopper. The granular material is placed in the hopper. By controlling the size of the auger and its rotation, a regulated volume of granular material can be delivered from the funnel stem into the container. Such a machine is shown in Fig 21.

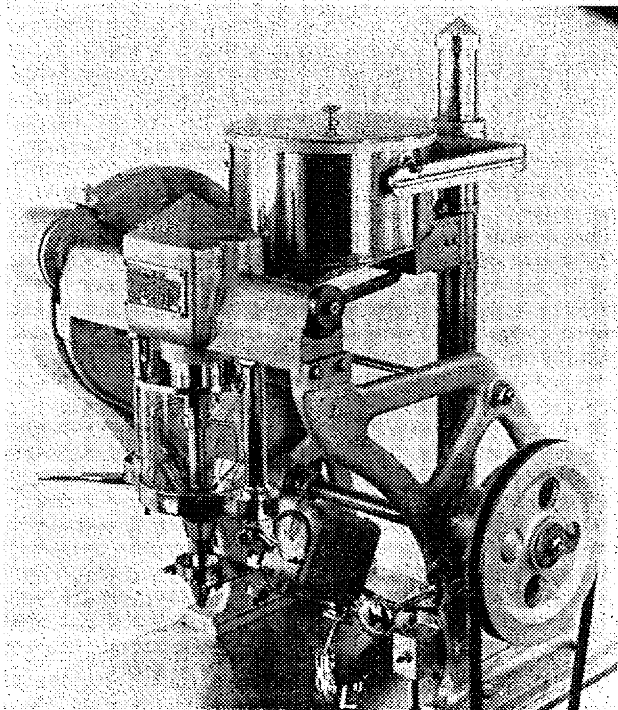


Fig 21. Auger-type powder filler (courtesy, Chase-Logeman).

Sealing

Ampuls—Filled containers should be sealed as soon as possible to prevent the contents from being contaminated by the environment. Ampuls are sealed by melting a portion of the glass neck. Two types of seals are employed normally: tip-seals (bead-seals) or pull-seals.

Tip-seals are made by melting enough glass at the tip of the neck of an ampul to form a bead and close the opening. These can be made rapidly in a high-temperature gas–oxygen flame. To produce a uniform bead, the ampul neck must be heated evenly on all sides. This may be accomplished by means of burners on opposite sides of stationary ampuls or by rotating the ampul in a single flame. Care must be taken to properly adjust the flame temperature and the interval of heating to obtain complete closing of the opening with a bead of glass. Excessive heating will result in the expansion of the gases within the ampul against the soft bead seal and cause a bubble to form. If it bursts, the ampul is no longer sealed; if it does not, the wall of the bubble will be thin and fragile. Insufficient heating will leave an open capillary through the center of the bead. An incompletely sealed ampul is called a "leaker."

Pull-seals are made by heating the neck of the ampul below the tip, leaving enough of the tip for grasping with forceps or other mechanical devices. The ampul is rotated in the flame from a single burner. When the glass has softened, the tip is grasped firmly and pulled quickly away from the body of the ampul, which continues to rotate. The small capillary tube thus formed is twisted closed.[1] Pull-sealing is slower, but the seals are more sure than tip-sealing. Fig 22 shows a machine combining the steps of filling and pull-sealing ampuls.

Powder ampuls or other types having a wide opening must be sealed by pull-sealing. Were these sealed by tip-sealing, the very large bead produced would induce glass strain with subsequent fracture at the juncture of the bead and neck wall. Fracture of the neck of ampuls during sealing also may occur if wetting of the necks occurred at the time of filling. Also, wet necks increase the frequency of bubble formation. If the product in the ampul is organic in nature, wet necks also will result in unsightly carbon deposits from the heat of sealing.

In order to prevent decomposition of a product, it is sometimes necessary to displace the air in the space above the product in the ampul with an inert gas. This is done by introducing a stream of the gas, such as nitrogen or carbon dioxide, during or after filling with the product. Immediately thereafter the ampul is sealed before the gas can diffuse to the outside. This process should be validated to ensure adequate displacement of air by the gas in each container.

Vials and Bottles—These are sealed by closing the opening with a rubber closure (stopper). This must be accomplished as rapidly as possible after filling and with reasoned care to prevent contamination of the contents. The large opening makes the introduction of contamination much easier

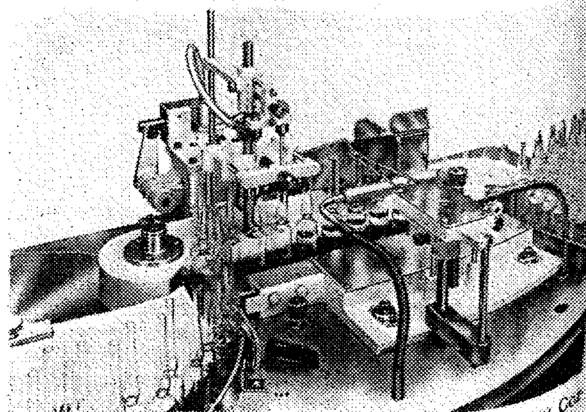


Fig 22. Automatic filling and pull-sealing of ampuls (courtesy, Cozzoli).

than with ampuls. Therefore, a covering should be provided for such containers except for the minimal time required for filling and for the actual introduction of the rubber closure. During the latter critical time the open containers should be protected from the ingress of contamination, preferably with a blanket of HEPA-filtered laminar airflow, as shown in Figs 10 and 19.

The closure must fit the mouth of the container snugly enough so that its elasticity will permit adjustment to slight irregularities in the lip and neck of the container. However, it must not fit so snugly that it is difficult to introduce into the neck of the container. Closures preferably are inserted mechanically using an automated process, especially with high-speed processing. To reduce friction so that the closure may slide more easily through a shute and into the container opening, the closure surfaces often are halogenated or treated with silicone. When the closure is positioned at the insertion site, it is mechanically pushed into the container opening (see Fig 23). When small lots are encountered, manual stoppering with forceps may be used, but such a process poses greater risk of introducing contamination than automated processes.

Rubber closures are held in place by means of aluminum caps. The caps cover the closure and are crimped under the lip of the vial or bottle to hold them in place (see Fig 5). The closure cannot be removed without destroying the aluminum cap; it is tamperproof. Therefore, an intact aluminum cap is proof that the closure has not been removed intentionally or unintentionally. Such confirmation is necessary to assure the integrity of the contents as to sterility and other aspects of quality.

The aluminum caps are so designed that the outer layer of double-layered caps, or the center of single-layered caps, can be removed to expose the center of the rubber closure without disturbing the band which holds the closure in the container. Rubber closures for use with intravenous administration sets often have a permanent hole through the closure. In such cases, a thin rubber disk overlayed with a solid aluminum disk is placed between an inner and outer aluminum cap, thereby providing a seal of the hole through the closure. These are called triple-layered aluminum caps.

Single-layered aluminum caps may be applied by means of a hand crimper known as the Fermpress (Suppliers: *West, Wheaton*). Double- or triple-layered caps require greater force for crimping; therefore, heavy-duty mechanical crimpers (see Fig 24) are required (Suppliers: *Bosch, Cozzoli, Perry, West, Wheaton*).

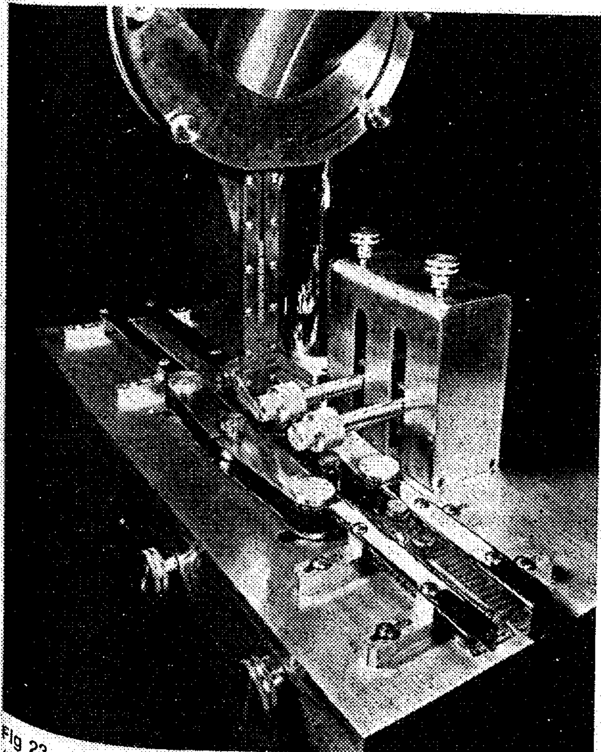


Fig 23. Mechanical device for inserting rubber closures in vials (courtesy, Perry).

Sterilization

Whenever possible, the parenteral product should be sterilized after being sealed in its final container (terminal sterilization) and within as short a time as possible after the filling and sealing have been completed. Since this usually involves a thermal process, due consideration must be given to the effect of the elevated temperature upon the stability of the product. Many products, both pharmaceutical and biological, will be affected adversely by the elevated temperatures required for thermal sterilization. Heat-labile products must, therefore, be sterilized by a nonthermal method, usually by filtration through bacteria-retaining filters. Subsequently, all operations must be carried out in an aseptic manner so that contamination will not be introduced into the filtrate. Colloids, oleaginous solutions, suspensions and emulsions that are thermolabile may require a process in which each component is sterilized separately and the product is formulated and processed under aseptic conditions.

The performance of an aseptic process is difficult and it has been accepted that currently, under the best controlled conditions, a sterility assurance level (SAL) of 10^{-3} is the maximum achievable. On the other hand, technical advances in aseptic processing, including improved automation, use of barrier systems, formulations to include antimicrobial effects and combinations of limited sterilization with aseptic processing, have improved the sterility-assurance levels achievable. Therefore, the successes realized should encourage continued efforts to improve the SAL achievable with aseptic processing. The importance of this is that for many drug solutions aseptic processing is the only method that can be considered for preparing a sterile product.

Nonthermal methods of sterilization, such as irradiation, have been proposed for consideration. However, since there is limited understanding of the molecular transformations that may occur in drug molecules and excipients under exposure to the high-energy levels of the process, extensive research will be required to develop the knowledge needed for an adequate evaluation.

Dry-heat sterilization may be employed for a few dry solids that are not affected adversely by the high temperatures and

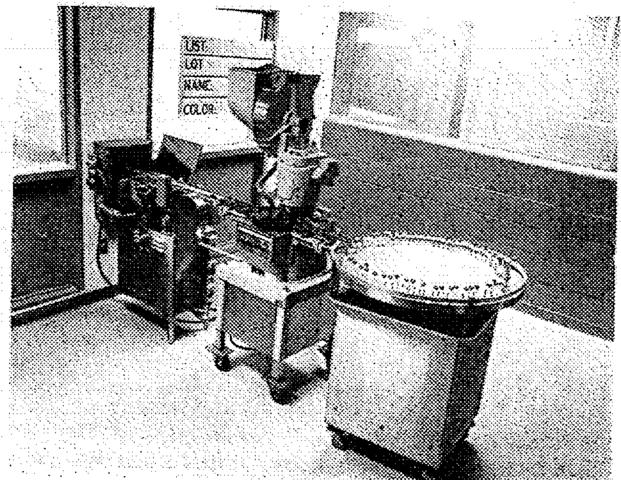


Fig 24. Applying aluminum caps to vials at the end of the process line (courtesy, Abbott).

for the relatively long heating period required. This method is applied most effectively to the sterilization of glassware and metalware. After sterilization, the equipment will be sterile, dry and, if the sterilization period is long enough, pyrogen-free.

Saturated steam under pressure (autoclaving) is the most commonly used and the most effective method for the sterilization of aqueous liquids or substances that can be reached or penetrated by steam. An SAL of 10^{-6} is readily achievable with terminal autoclaving of a thermally stable product. This is a 1000-fold improvement over the currently accepted level for aseptic processing. However, it needs to be noted that for terminal sterilization, as with autoclaving, the SAL is based upon an evaluation of the lethality of the process, ie, of the probable number of viable microorganisms remaining in product units. However, for aseptic processing, the components used have been sterilized by a validated process and the SAL is based upon an evaluation of the probable number of product units that have been contaminated during the process. This difference does not alter the outcome but only the basis for evaluating the probable SAL.

Figure 25 shows liter containers of solution being loaded into an autoclave for sterilization. Since the temperature employed in an autoclave is lower than that for dry-heat sterilization, equipment made of materials such as rubber and polypropylene may be sterilized if the time and temperature are controlled carefully. As mentioned previously, some injections will be affected adversely by the elevated temperature required for autoclaving. For some products, such as Dextrose Injection, the use of an autoclave designed to permit a rapid rise to sterilizing temperature and rapid cooling with water spray after the sterilizing hold-period will make it possible to use this method. It is ineffective in anhydrous conditions, such as within a sealed ampul containing a dry solid or an anhydrous oil. Other products that will not withstand autoclaving temperatures may withstand marginal thermal methods such as tyndallization or inspissation. These methods may be rendered more effective for some injections by the inclusion of a bacteriostatic agent in the product.

It should be obvious that all materials subjected to sterilization must be protected from subsequent contamination to maintain their sterile state. Therefore, they must be wrapped or covered so that microorganisms may not gain access when removed from the autoclave. Equipment and supplies are wrapped most frequently with paper and tied or sealed with special autoclave tape. The wrapping must permit penetration of steam during autoclaving but screen out microorganisms when dry. A double wrapping with lint-free parchment paper designed for such use is probably best. Synthetic fiber cloth such as nylon or Dacron also may be used for the inner wrapping. The openings of equipment subjected to dry-heat sterilization are often covered with silver–aluminum foil or with metal or glass covers. Cellulose wrapping materials are affected adversely by the high temperatures of dry-heat sterilization.

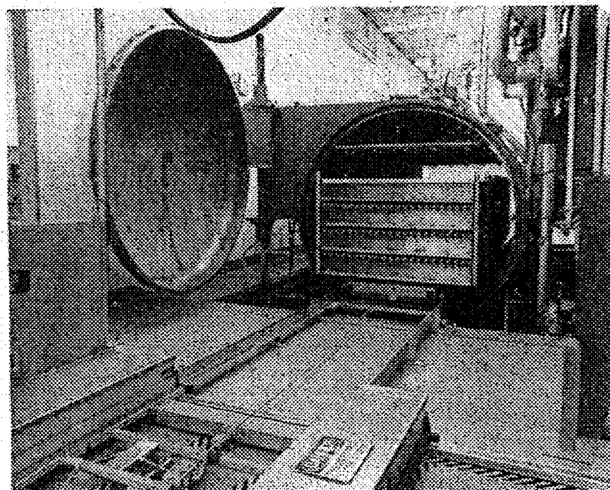


Fig 25. Large autoclave being loaded with liter bottles of parenteral solutions (courtesy, Abbott).

The effectiveness of any sterilization technique must be proved (validated) before it is employed. Since the goal of sterilization is to kill microorganisms, the ideal indicator to prove the effectiveness of the process is a resistant form of an appropriate microorganism, normally resistant spores (a biological indicator or BI). Therefore, during validation of a sterilization process, BIs of known resistance are used and numbers in association with physical-parameter indicators, such as recording thermocouples. Once the lethality of the process is established in association with the physical measurements, the physical measurements can be used for subsequent monitoring of in-use processes without the BIs. Eliminating the use of BIs in direct association with human-use products is appropriate because of the ever-present risk of an undetected, inadvertent contamination of a product or the environment.

The commercial suppliers of BIs have improved the resistance evaluation and lot-to-lot reliability of their products so that the information provided with each lot of BIs is generally reliable and useful in validation procedures.

In addition to the data printout from thermocouples, sometimes other physical indicators are used, such as color-change and melting indicators, to give visual indication that a package or truckload has been subjected to a sterilization process. Such evidence can become a part of the batch record to confirm that sterilization was accomplished.

Further details concerning methods of sterilization and their application will be found in Chapter 84. In addition, the USP provides suggestions concerning the sterilization of injections and related materials.

Freeze-Drying

Freeze-drying (lyophilization) is a process of drying in which water is sublimed from the product after it is frozen.[24] The particular advantages of this process are that biologicals and pharmaceuticals which are relatively unstable in aqueous solution can be processed and filled into dosage containers in the liquid state, taking advantage of the relative ease of processing a liquid. They can be dried without elevated temperatures, thereby eliminating adverse thermal effects, and stored in the dry state in which there are relatively few stability problems.

Further advantages are that these products are often more soluble and/or more rapidly soluble, dispersions are stabilized throughout their shelf life and products subject to degradation by oxidation have enhanced stability because the process is carried out in a vacuum.

However, the increased time and handling required for processing and the cost of the equipment limit the use of this process to those products which significantly have enhanced stability if stored in the dry state.

The fact that ice will sublime at pressures below 3 torr has been a long-established laboratory principle (see Chapter 19). The extensive program for freeze-drying human plasma during World War II provided the impetus for the rapid development of the process.

Freeze-drying essentially consists of

1. Freezing an aqueous product at a temperature below its eutectic temperature.
2. Evacuating the chamber, usually below 0.1 torr (100 μm Hg).
3. Subliming ice on a cold condensing surface at a temperature below that of the product, the condensing surface being within the chamber or in a connecting chamber.
4. Introducing heat to the product under controlled conditions, thereby providing energy for sublimation at a rate designed to keep the product temperature below its eutectic temperature.

Figure 26 shows such a system. The product may be fr

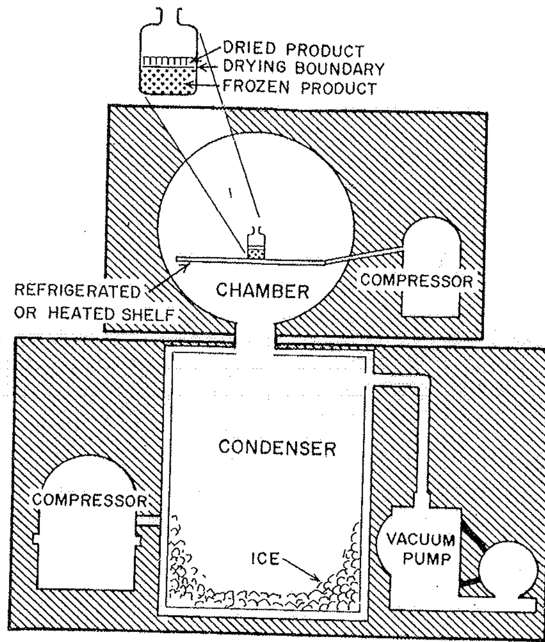


Fig 26. Essential components of a freeze-drying system.

zen on the shelf in the chamber by circulating refrigerant (usually Freon, ammonia or ethylene glycol) from the compressor through pipes within the shelf. After freezing is complete, which may require several hours, the chamber and condenser are evacuated by the vacuum pump, the condenser surface having been chilled previously by circulating refrigerant from the large compressor.

Heat then is introduced from the shelf to the product by electric resistance coils or by circulating hot water, silicone or glycol. The process continues until the product is dry (usually 1% or less moisture), leaving a sponge-like matrix of the solids originally present in the product, the input of heat being controlled so as not to degrade the product.

For most pharmaceuticals and biologicals the liquid product is sterilized by filtration and then filled into the dosage container aseptically. The containers must remain open during the drying process to allow water vapor to escape; therefore, they must be protected from contamination during transfer from the filling area to the freeze-drying chamber, while in the freeze-drying chamber and at the end of the drying process until sealed.

The chambers may be equipped with hydraulic or rubber diaphragm internal-stoppering devices designed to push slotted rubber closures into the vials to be sealed while the chamber is still evacuated, the closures having been partially inserted immediately after filling so that the slots were open to the outside.

If internal stoppering is not available or containers such as ampuls are used, filtered dry air or nitrogen must be introduced to the chamber at the end of the process to establish atmospheric pressure. Then the containers must be removed and sealed under aseptic conditions. If the product is very sensitive to moisture, the environmental humidity also must be controlled until it is sealed.

Factors Affecting the Process Rate—The greater the depth of the product in the container, the longer will be the drying process. Therefore, a product to be frozen by placing the container on a refrigerated shelf (plug freezing) should be filled to a planned, limited depth. If a large volume of solution must be processed, the surface area may be increased and the depth decreased by freezing the solution on a slant or while rotating the container on an angle (shell freezing) in a liquid refrigerant bath, such as dry ice and alcohol.

The actual driving force for the process is the vapor pressure differential between the vapor at the surface where drying of the product is occurring (the drying boundary) and that at the surface of the ice on the condenser. The latter is determined by the temperature of the condenser as modified by the insulating effect of the accumulated ice. The former is determined by a number of factors, including:

1. The rate of heat conduction through the container and the frozen material, both usually relatively poor thermal conductors, to the drying boundary while maintaining all of the product below its eutectic temperature.
2. The impeding effect of the increasing depth of dried, porous product above the drying boundary.
3. The temperature and heat capacity of the shelf itself.

This may be visualized by referring to Fig 26.

The passageways between the product surface and the condenser surface must be wide open and direct for effective operation. Therefore, the condensing surfaces in large freeze-driers are usually in the same chamber as the product. Evacuation of the system is necessary to reduce the impeding effect that collisions with air molecules would have on the passage of water molecules. However, the residual pressure in the system must be greater than the vapor pressure of the ice on the condenser or the ice will be vaporized and pulled into the pump, an event detrimental to most pumps.

The amount of solids in the product, their particle size and their thermal conductance will affect the rate of drying. The more solids present, the more impediment will be provided to the escape of the water vapor. The smaller the particle size, particularly the crystal size of the ice, the faster the drying generally will be. The poorer the thermal conducting properties of the solids in the product, the slower will be the rate of heat transfer through the frozen material to the drying boundary.

The rate of drying is essentially slow, most often requiring 24 hours or longer for completion. The actual time required, the rate of heat input and the product temperatures that may be used must be determined for each product and then reproduced carefully with successive processes.

Factors Affecting Formulation—The active constituent of many pharmaceutical products is present in such a small quantity that if freeze-dried alone its presence would be hard to detect visually. Therefore, excipients often are added to increase the amount of solids.

Some consider it ideal for the dried-product plug to occupy essentially the same volume as that of the original solution. To achieve this, the solids content of the original product must be between approximately 5 and 25%. Among the substances found most useful for this purpose, usually as a combination, are sodium or potassium phosphates, citric acid, tartaric acid, gelatin and carbohydrates such as dextrose, mannitol and dextran.

Each of these substances contributes appearance characteristics of the plug, such as whether dull and spongy or sparkling and crystalline, firm or friable, expanded or shrunken and uniform or striated. Therefore, the formulation of a product to be freeze-dried must include consideration not only of the nature and stability characteristics required during the liquid state, both freshly prepared and when reconstituted before use, but the characteristics desired in the dried plug.

Modifications in the Process and Equipment—In some instances a product may be frozen in a bulk container or in trays rather than in the final container and then handled as a dry solid. This may be desirable when large volumes of a product are processed, but the risk of contamination from the environment is high.

When large quantities of material are processed it may be desirable to use ejection pumps in the equipment system. These draw the vapor into the pump and eject it to the outside, thereby eliminating the need for a condensing surface. Such pumps are expensive and usually practical only in large installations.

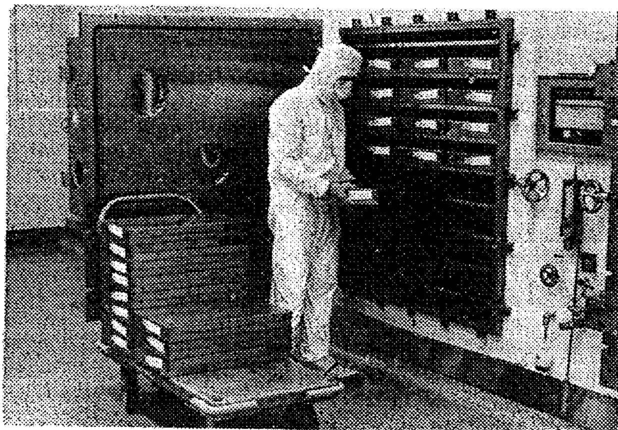


Fig 27. Aseptic loading of freeze-drier (courtesy, Upjohn).

Available freeze-driers (Suppliers: *Edwards, Finn-Aqua, ETS, Hull, NRC, Stokes, Virtis*) range in size from small laboratory units to large industrial models such as the one shown in Fig 27. Their selection requires consideration of such factors as

The tray area required.
The volume of water to be removed.
Whether or not aseptic processing will be involved.
Is internal stoppering required?
Will separate freezers be used for initial freezing of the product.
The degree of automatic operation desired.

Other factors involved in the selection and use of equipment are considered in the literature.[25]

Freeze-drying is now being used for research in the preservation of human tissue and is finding increasing application in the food industry. Progress on new developments is being made in both the process and the equipment.[26]

Quality Assurance and Control

The importance of undertaking every possible means to assure the quality of the finished product cannot be overemphasized. Every component and step of the manufacturing process must be subjected to intense scrutiny to be confident that quality is attained in the finished product. The responsibility for achieving this quality is divided appropriately in concept and practice into Quality Assurance (QA) and Quality Control (QC). QA relates to the studies made and the plans developed for assuring quality of a product prospectively. QC embodies the carrying out of these plans during production and includes all of the tests and evaluations performed to be sure that quality has been achieved in a specific lot of product.

The principles for achieving quality are basically the same for the manufacture of any pharmaceutical. These are discussed in Chapter 39. During the discussion of the preparation of injections, mention was made of numerous quality requirements for components and manufacturing processes. Here, only selected tests characteristically required before a finished product is released will be discussed briefly, including sterility, pyrogen and particulate tests.

Sterility Test

All lots of injections in their final containers must be tested for sterility. The USP prescribes the requirements for this test for official injections. The FDA uses these requirements as a guide for testing unofficial sterile products. The official test has acknowledged limitations in the information that it can provide, particularly those limitations inherent in microbiological procedures. Therefore, it should be noted that this test is not intended as a thoroughly evaluative test for a product subjected to a sterilization method of unknown effectiveness. It is intended primarily as a check test on the probability that a previously validated sterilization procedure has been repeated, or to give assurance of its continued effectiveness. A discussion of sterility testing is given in Chapter 84.

In the event of a sterility-test failure, the immediate issue concerns whether the growth observed came from viable microorganisms in the product (true contamination) or from adventitious contamination during the testing (a false positive). The USP does permit a retest but the position of the FDA is that retest results are only valid if persuasive evidence exists that the cause of the initial sterility-test failure resides in the laboratory. Therefore, a thorough investigation must be launched to support the justification for performing the retest and assessing the validity of the retest results relative to release of the lot of product.

It should be noted that a "lot" with respect to sterility testing is that group of product containers which has been subjected to the same sterilization procedure. For containers of a product which have been sterilized by autoclaving, for example, a lot would constitute those processed in a particular sterilizer cycle. For an aseptic filling operation, a lot would constitute all of those product containers filled during a period when there was no change in the filling assembly or equipment and which is no longer than one working day or shift.

Pyrogen Test

The USP evaluates the presence of pyrogens in parenteral preparations by a qualitative fever response test in rabbits, the Pyrogen Test (Section ⟨151⟩), and by the Bacterial Endotoxins Test (Section ⟨85⟩). These two USP tests are described in Chapter 30. Rabbits are used as test animals in Section ⟨151⟩ because they show a physiological response to pyrogenic substances similar to that by man. While a minimum pyrogenic dose (MPD), the amount just sufficient to cause a positive USP Pyrogen Test response, sometimes may produce uncertain test results, a content equal to a few times the MPD will leave no uncertainty. Therefore, the test is valid and has continued in use since introduced by Seibert in 1923. It should be understood that not all injections may be subjected to the rabbit test since the medicinal agent may have a physiological effect on the test animal such that any fever response would be masked.

The *Bacterial Endotoxins Test* is an *in vitro* test based on the formation of a gel or the development of color in the presence of bacterial endotoxins and the lysate of the amebocytes of the horseshoe crab (*Limulus polyphemus*). The *Limulus Amebocyte Lysate* (LAL) test, as it also is called, is a biochemical test performed in a test tube, and is simpler, more rapid and of greater sensitivity than the rabbit test.[27] Although it detects only the endotoxic pyrogens of gram-negative bacteria, these are the most prominent environmental microbial contaminants likely to invade sterile products. The test also has been automated.[28]

The LAL test is a semiquantitative test. To provide standardization for the test, the USP has established a reference endotoxin against which lots of the lysate are standardized.

Thus, the sensitivity of the lysate is given in terms of endotoxin units (EU). Most USP injections now have been given limits in terms of EUs (eg, Bacteriostatic Sodium Chloride Injection, 1.0 EU/mL) as another measure of the purity of the product.

Particulate Evaluation

Particulate matter in parenteral solutions long has been recognized as unacceptable since the user could be expected to conclude that the presence of visible "dirt" would suggest that the product is of inferior quality. Today, it is recognized that the presence of particles in solution, particularly if injected intravenously, can be harmful. While data defining the extent of risk and the effects produced still are limited, it has been shown that particles of lint, rubber, insoluble chemicals and other foreign matter can produce emboli in the vital organs of animals and man.[29] Further, it has been shown that the development of infusion-phlebitis may be related to the presence of particulate matter in intravenous fluids.[30]

The particle size of particular concern has not been clearly delineated, but it has been suggested that since erythrocytes have a diameter of approximately 4.5 μm, particles of more than 5 μm should be the basis for evaluation. This is a considerably smaller particle than can be seen with the unaided eye; approximately 50 μm is the lower limit unless the Tyndall effect is used whereby particles as small as 10 μm can be seen by the light scattered from them.

The USP specifies that good manufacturing practice requires that each final container of an injection be subjected individually to a visual inspection and that containers in which visible particles can be seen should be discarded. This 100% inspection of a lot of product is designed to prevent the distribution and use of parenterals which contain particulate matter that may be harmful psychologically or organically to the participant. Therefore, all of the product units from a production line currently are being inspected individually by human inspectors under a good light, baffled against reflection into the eye and against a black-and-white background. This inspection is subject to the limitation of the size of particles that can be seen, the variation of visual acuity from inspector to inspector, their emotional state, eye strain, fatigue and other personal factors that will affect what is seen. However, it does provide a means for eliminating the few units which normally contain visible particles. Slow progress has been made on developing equipment for automated inspection of product containers.

Since it is recognized that visual inspection will not detect the presence of particles smaller than approximately 50 μm in size, the USP has established a microscopic test method for identifying particles in large-volume intravenous solutions and has set limits of not more than 50 particles/mL of 10 μm and larger in size and not more than 5 particles/mL of 25 μm and larger in size. This method consists essentially of filtering a measured sample of solution through a membrane filter under ultraclean conditions and then counting the particles on the surface of the filter using oblique light, under a microscope, at both 40x and 100x magnification. These standards are being met readily by the large-volume parenteral solutions currently being manufactured in the US.

More recently the USP established standards for small-volume parenterals to be given intravenously, using an electronic instrument that counts and measures the size of particles by means of a shadow cast by the particle as it passes through a high-intensity light beam (Suppliers: *Climet, HIAC*). The limits prescribed are not more than 10,000 particles/container of ≥ 10 μm in size and not more than 1000 particles/container ≥ 25 μm in size. These specifications were developed on the premise that as many as five such products may be added to a 1-L bottle of a large-volume parenteral and five products should not contribute more than the overall limits of particles prescribed for a large-volume parenteral. Whether or not these standards are realistic toxicologically has not been established; rather, the objective of the compendium is to establish specification limits that would encourage the preparation of clean parenteral solutions, particularly for those to be given intravenously.

It also should be realized that administration sets and the techniques used in the hospital for preparing and administering intravenous infusion fluid may introduce substantial amounts of particulate matter into an otherwise clean solution. Therefore, the pharmaceutical manufacturer, the administration set manufacturer, the hospital pharmacist, the nurse and the physician must share responsibilities for making sure that the patient receives a clean intravenous injection.

The USP methods for counting and sizing particulate matter in intravenous solutions are not the only methods available for such determinations. A number of electronic particle counters are available that use the light-scattering principle to count particles in a liquid sample (Suppliers: *Climet, Met One, HIAC/Royco*). There also is an instrument available which counts particles and sizes them by measuring the effect on the resistance between two electrodes as the particles pass between them (Supplier: *Coulter*). It is obvious that only the visual inspection can be used for in-line evaluation of every container produced commercially. All of these methods require very stringent ultraclean preparation techniques to assure reasonable accuracy in counting and sizing only the particles in the solution, rather than those that may have been introduced inadvertently during the sample preparation or the testing procedure. Further, these test procedures are destructive and, therefore, can be performed only on samples of the production lot. Further information may be found in a review article.[31]

Leaker Test

Ampuls that have been sealed by fusion must be subjected to a test to determine whether or not a passageway remains to the outside; if so, all or a part of the contents may leak to the outside and spoil the package, or microorganisms or other contaminants may enter. Changes in temperature during storage cause expansion and contraction of the ampul and contents, and will accentuate intechange if a passageway exists, even if microscopic in size.

This test usually is performed by producing a negative pressure within an incompletely sealed ampul while the ampul is entirely submerged in a deeply colored dye solution. Most often, approximately 1% methylene blue solution is employed. The test may be performed by subjecting the ampuls to a vacuum in a vacuum chamber, the ampuls being submerged in a dye bath throughout the process. Another procedure frequently employed is to simply autoclave the ampuls in a dye bath. A modification of this is to remove them from the autoclave while hot and quickly submerge them in a cool bath of dye solution. After carefully rinsing the dye solution from the outside, color from the dye will be visible within a leaker. Leakers, of course, are discarded.

Vials and bottles are not subjected to a leaker test because the sealing material (rubber stopper) is not rigid. Therefore, results from such a test would be meaningless. However, evacuated bottles containing a liquid may be checked for a sharp "click" sound produced when struck with an implement such as a rubber mallet or the ball of the hand. However, assurance of container-closure sealing integrity should be an integral part of product development by developing specifications for the fit of the closure in the neck of the container, the physical characteristics of the closure, the need for lubrication of the closure and the capping pressure.

Safety Test

The National Institutes of Health requires of most biological products routine safety testing in animals. Under the Kefauver-Harris Amendments to the Federal Food, Drug, and Cos-

metic Act, most pharmaceutical preparations are now required to be tested for safety. Because it is entirely possible for a parenteral product to pass the routine sterility test, pyrogen test and chemical analyses and still cause unfavorable reactions when injected, a safety test in animals is essential to provide additional assurance that the product does not have unexpected toxic properties. Safety tests in animals are discussed in detail in the USP.

Packaging and Labeling

A full discussion of the packaging of parenteral preparations is beyond the scope of this text. It is essential, of course, that the packaging should provide ample protection for the product against physical damage from shipping, handling and storage as well as protecting light-sensitive materials from ultraviolet radiation. An extensive review of this subject has been published.[32]

Packaging—The USP includes certain requirements for the packaging and storage of injections, as follows:

1. The volume of injection in single-dose containers is defined as that which is specified for parenteral administration at one time and is limited to a volume of 1 L.
2. Parenterals intended for intraspinal, intracisternal or peridural administration are packaged only in single-dose containers.
3. Unless an individual monograph specifies otherwise, no multiple-dose container shall contain a volume of injection more than sufficient to permit the withdrawal and administration of 30 mL.
4. Injections packaged for use as irrigation solutions or for hemofiltration or dialysis or for parenteral nutrition are exampt from the foregoing requirements relating to packaging. Containers for injections packaged for use as hemofiltration or irrigation solutions may be designed to empty rapidly and may contain a volume in excess of 1 L.
5. Injections intended for veterinary use are exempt from the packaging and storage requirements concerning the limitation to single-dose containers and to volume of multiple-dose containers.

Labeling—The labeling of an injection must provide the physician or other user with all of the information needed to assure the safe and proper use of the therapeutic agent. Since all of this information cannot be placed on the immediate container and be legible, it may be provided on accompanying printed matter. General labeling requirements for drugs are discussed in Chapter 110.

A restatement of the labeling definitions and requirements of the USP for Injections is as follows:

The term "labeling" designates all labels and other written, printed or graphic matter upon an immediate container or upon, or in, any package or wrapper in which it is enclosed, with the exception of the outer shipping container. The term "label" designates that part of the labeling upon the immediate container.

The label states the name of the preparation, the percentage content of drug of a liquid preparation, the amount of active ingredient of a dry preparation, the volume of liquid to be added to prepare an injection or suspension from a dry preparation, the route of administration, a statement of storage conditions and an expiration date. Also, the label must indicate the name of the manufacturer or distributor and carry an identifying lot number. The lot number is capable of providing access to the complete manufacturing history of the specific package, including each single manufacturing step.

The container label is so arranged that a sufficient area of the container remains uncovered for its full length or circumference to permit inspection of the contents.

The label must state the name of the vehicle and the proportions of each constituent, if it is a mixture; the names and proportions of all substances added to increase stability or usefulness and the expiration date where required by the individual monograph.

Preparations labeled for use as dialysis, hemofiltration or irrigation solutions must meet the requirements for Injections other than those relating to volume and also must bear on the label statements that they are not intended for intravenous injection.

Injections intended for veterinary use are so labeled.

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