A NEW APPROACH TO STERILITY TESTING

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In carrying out any aseptic operation, extraneous contamination may be introduced from the environment or from the operators, and many and varied precautions are practised in efforts to minimise it. The purpose of this paper is to describe a sealed screen technique which has proved successful in enhancing the precision of sterility testing; it can also be applied to other difficult small scale operations.

Such operations usually require specially designed "sterile" rooms which can be thoroughly disinfected before each work session. The rooms are often provided with airlocks and there is usually a flow of sterile air to keep the level of aerial contamination as low as possible. However, it must be accepted that complete elimination of all airborne contamination in a room occupied by even one or two operators is unattainable, in spite of the most careful preliminaries of "scrubbing up" and the wearing of sterilised gowns and other coverings. Therefore, as further precautions for insulating the work from such contamination and so preventing access of organisms to the material being handled, screens of various designs are employed, combined with careful flaming techniques, studied motions and minimum exposure of materials and containers to the open atmosphere.

Such methods used properly can result in negligible adventitious contamination. Nevertheless they are not absolute; the degree of contamination introduced is variable and depends on the sustained concentration and skill of the operators and of the difficulty of the operations concerned. For these reasons a detailed training scheme for all operators should be followed, such as that proposed by Coulthard[1], and the techniques employed need to be continually checked, as emphasised by Sykes[2], to assess the level of contamination introduced and to maintain the necessary high degree of asepsis.

The enclosed screen technique described below is an attempt to approach more closely and with greater certainty the absolute standard of asepsis required in sterility testing. It can be used for many other aseptic manipulations, but it is not universally applicable, owing to certain limitations mentioned later. The actual manipulations within the screen are more difficult and possibly slower to carry out because of restriction of movements imposed by the enclosed design. But set against these disadvantages are the distinct advantages of being able to dispense with sterile rooms and the usual scrubbing up and sterile dressing procedures. Consequently there is a considerable saving of time in the preliminary preparations resulting in even greater productivity where large numbers of tests for sterility have to be carried out. Moreover, the greater certainty of the method practically eliminates repeat tests made necessary from adventitious contamination introduced during testing.

THE SEALED SCREEN

Design and construction. The screen consists basically of a sheet-metal sealed box, with a large removable hatch, and fitted with long-sleeved rubber gauntlets. The size can be varied according to requirements but it is limited by the reach of the arms within the screen, unless reaching tools, tongs, etc., are provided. The model illustrated in Figure 1 is approximately 2 ft. 6 in. long, 2 ft. deep and 1 ft. 6 in. high. It is built on an angle-iron frame to such a height as will enable an operator to sit

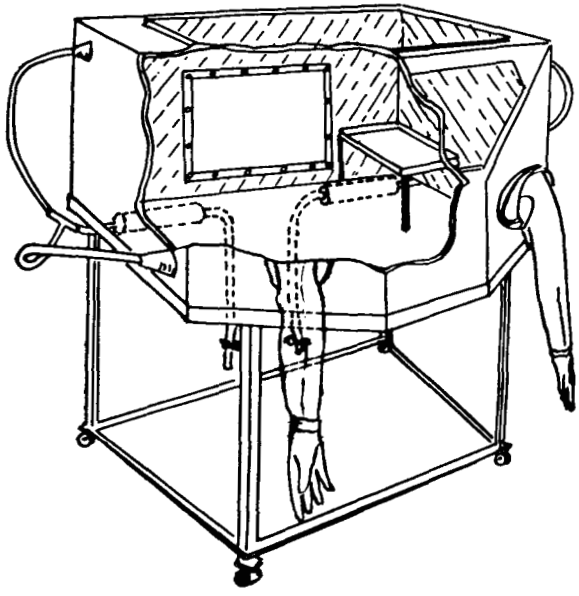

FIG. 1. Diagram of aseptic screen.

comfortably at the screen; it is mounted on wheels for mobility. All the joints of the screen must be well sealed with solder or other sealing compound so that it is practically gas-tight. It is provided with two perspex windows, one in the top to admit light and another in the sloping front to enable the operator to see inside. The removable hatch, through which the screen is loaded and unloaded, is built in the back; it is fixed in position by means of a number of wing nuts and sealed with a sponge rubber gasket. A removable tray is provided to take the remnants of samples, used syringes and other discarded materials. At diagonally opposite corners of each end of the screen are fixed short metal tubes joined by rubber tubing to air filters clipped to the back of the screen, the pair of tubes at one end being connected to one filter and those at the other end to a second filter. The filters are made from glass or metal tube about 1 ft. long and $1\frac{1}{2}$ in. in diameter and packed with non-absorbent cotton wool. A screw clip on a short length of rubber tubing at the distal end of each filter allows it to be closed off as required.

The screen is also provided with two oval, flanged arm holes to which the rubber gauntlet gloves are sealed; the oval shape allows a certain lateral arm movement. The holes are set on the angle faces of the front to give the most comfortable and efficient working position for the operator. The gauntlet arms are made of heavy gauge rubber and are wide enough at one end to fit the flange of the arm holes, to which they are sealed by means of a sponge rubber gasket and a metal band fitted with a tightening screw. The gauntlets are tapered down their length so that at the other end they can be sealed to the wrists of rubber gloves. Heavy domestic grade gloves are necessary; those of surgical quality are not sufficiently robust for this purpose as they are too easily ruptured. It is advisable to reinforce the rubber joints with an elastic adhesive band.

From the foregoing description it is seen that when the door is in position and the air filters are sealed off, the whole screen is, for all practical purposes, gas tight. Within the screen, any normal hand and finger manipulations can be carried out by an operator who is himself external to the system.

Operation of the screen. The principle of the operation of the screen is that the whole of the contents, including the surfaces of apparatus, bottles and other containers placed in the screen, can be sterilised in a suitable gaseous atmosphere—ethylene oxide is used for this purpose—after which any aseptic manipulations can be carried out within the screen without any possibility of contamination from the environment or operator.

Because the sterilising agent is a gas, it is obvious that it must not gain access to any of the materials being handled, neither must it be in contact with liquids in which it may dissolve or react. Therefore, all samples and culture media must be in sealed containers. Screw-capped containers for culture media are not novel; they were advocated some years ago[3], and they are in common bacteriological use to-day. For anaerobic media they are advantageous as the seal reduces to a minimum the rate of diffusion of oxygen into the sterilised media. For aerobic media an adequate air space must be left. In practice, a space equivalent to one-quarter of the total capacity of the container allows the free growth of strictly aerobic organisms.

The test samples, the necessary culture media, syringes and any apparatus required for measuring, weighing or redistributing into other containers, are first loaded into the screen. All requirements should be known and remembered at this stage, as any item forgotten cannot be put into the screen once it has been sterilised. Syringes, pipettes and other apparatus required for handling samples can be sterilised *in situ*. It is advisable, however, to treat them in the autoclave beforehand in suitable containers, so that the sterilising gas has then only to deal with a superficial surface infection and is not required to disinfect the inner surfaces of needles, syringe barrels and pipettes where diffusion of the gas may not always be adequate to sterilise in the time allowed.

A 12·5 per cent. (v/v) concentration of gaseous ethylene oxide is used for sterilising. Since it is liquid at temperatures below about 10° C.

it is most conveniently handled in this form. Therefore, the calculated quantity of the liquid in a chilled, screw-capped bottle is placed in the screen and the hatch is fastened in position. Immediately after closing the screen, the liquid ethylene oxide is poured on the floor of the screen. It immediately evaporates and the slight increase in pressure created in the screen is allowed to escape through the filters. When the balance is restored, the filters are sealed off by means of the screw clips and the sterilisation process is allowed to proceed overnight for 16 to 24 hours. After completion of this period the screen is flushed for about half an hour with sterile air introduced through one of the filters. The flushing must be such that it removes virtually all of the ethylene oxide gas. Subsequently, manipulations of any sort, including the opening of the bottles of culture media, can be carried out in the screen with impunity.

Two points should be borne in mind in connection with the sterilisation procedure. First, there is a small loss of ethylene oxide from the system when the excess pressure in the screen is allowed to escape. Secondly, there is a small loss by absorption of the gas by the rubber of the gauntlets and gloves. These losses can easily be balanced by including a slight excess of ethylene oxide in the first place, a 10 per cent. excess is adequate.

One objection to the technique is that owing to the enclosed nature of the screen a heavy contamination in one batch of a product, or even in one container of a batch, could easily cross-infect tests on other materials being examined at the same time. However, before materials are submitted for testing, they have usually been processed in such a way as to be reasonably certain that they are sterile. Most groups of samples examined are, in fact, sterile, or the contamination encountered is sufficiently light to render spread of infection highly improbable. The few products in which a heavy contamination may arise, due to the nutrient properties of the solution and the absence of a bacteriostatic agent, are known to the experienced operator and special isolating precautions can be taken. The simplest procedure with suspect material, including that which may have shown a contamination in a previous test, is either to put the tests on separately or to perform them last of a series in the screen.

The sterilising gas. Several gases might conceivably be chosen as suitable sterilising agents, but the majority have physical or chemical disadvantages. Thus chlorine and sulphur dioxide would attack the metal surfaces, and formaldehyde is difficult to flush out of the screen. For these reasons, ethylene oxide was chosen.

Ethylene oxide can be used as a sterilising agent both in solution[4], and in the gaseous phase. Its disinfectant action in the gaseous phase was first recorded in an American Patent[5] in 1936, since which time several further publications have appeared on the subject. It has been reported that glass, metal and paper surfaces and dry or wet rags infected with *Bacillus anthracoides* were easily disinfected in 8 hours by a concentration of 200 mg. per litre[6], and soils of different types were sterilised by the gas in periods ranging between 2 hours and 6 hours[7]. It is asserted also[8] that blankets and linens, soiled or laundered, are completely sterilised overnight by 10 per cent. of ethylene oxide in carbon dioxide. A useful review

of the subject was presented by Phillips and Kaye[9] who in subsequent papers[10,11] discussed the influence of time, concentration, temperature and moisture on the rate of disinfection of the spores of *Bacillus globigii*.

In our experience with the screen, we have found that a 12·5 per cent. gaseous concentration will sterilise glass and metal surfaces in 16 hours, provided the surfaces are clean and dry. If they carry any grease films or dried broth residues, etc., organisms may be protected from the action of the ethylene oxide and so remain viable. With the proper precautions taken, ethylene oxide has been uniformly successful in daily use for a period of over 3 years.

Attention must be drawn to certain of the undesirable properties of ethylene oxide. It boils at 10·7° C. and its vapour is toxic when inhaled. It is also explosive in mixtures with air between 3 per cent. and 100 per cent., but carbon dioxide quenches its explosiveness. Finally, in contact with the skin it can cause severe reactions. The amount absorbed by rubber is significant in this respect, so that unless due precautions are taken the operators are liable to suffer eruptions on the hands and arms. This has been studied in detail by Royce and Moore[12], who found that the danger could be obviated by adequately airing the gloves by hanging them in free air (see Fig. 1) for a minimum of 1 hour after flushing the screen.

Discussion

The screen described can be applied to all types of tests for sterility where the material is packed in gas-tight containers. Thus it cannot be used in testing surgical dressings. On the other hand, it is valuable for carrying out many complicated aseptic manipulations with a greater degree of certainty. In this connection it can be used to advantage for carrying out the Davies and Fishburn[13] filtration test technique which otherwise is subject to the hazard of accidental aerial contamination. It is also useful for such operations as breaking down quantities of sterile bulk solids into smaller containers where perhaps weighings are involved, and for dispensing sterile media or other solutions which are heat labile or require the mixing of a number of previously sterilised constituents.

The principal virtue of the technique, however, is that in sterility testing it eliminates almost completely the risk of infection from outside sources, so that any growth occurring in a test must almost certainly have originated from the material under examination. The question does not arise, therefore, whether a contamination might have been introduced during testing. To illustrate the value and reliability of the technique in sterility testing, it has been subject to control testing since the screens were put into operation in 1951. A "control" test is one carried out under normal conditions but with test material such as water, saline or sodium chloride sterilised in their appropriate containers by a reliable process in the laboratory. They represent tests on the "bulk" stage of a manufactured product and on the "final container" stage in which 20 containers are examined in each test. Of some 800 such control tests carried out during the period, only one was contaminated.

SUMMARY

1. A sealed screen is described. It consists of a sheet-metal, sealed box with a large removable hatch and is fitted with long-sleeved rubber gauntlets.

2. The contents and the surfaces within the screen are sterilised by gaseous ethylene oxide after which any aseptic operations may be carried out free of contamination from environment or operator.

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DISCUSSION

The paper was presented by MR. A. ROYCE.

DR. H. S. BEAN (London) said that if a suitable technique on the lines proposed could be developed it would considerably simplify sterility testing. It would be interesting to learn how the authors obtained the sterile air which was passed into the screen. He asked why the authors resorted to a rather hazardous material such as ethylene oxide since there were other chemicals available. Aerosols which functioned in low concentrations would not be as dangerous. In his experience screw cap containers were not ideal for growing cultures, and he sometimes failed to grow *B. subtilis* in such bottles.

DR. R. M. SAVAGE (Barnet) said that it should be emphasised that what the test did was to reduce the contamination to a very low level and not to eliminate it altogether. In the last paragraph of the discussion it was stated that "in sterility testing it eliminates almost completely the risk of infection from outside sources . . ." but there followed the statement that "any growth occurring in a test must certainly have originated from the material under examination." It would be preferable to see the word "almost" inserted before "certainly" because the possibility of contamination had not been reduced to zero. One would also have liked to see some evidence that material which was very lightly contaminated had not had that light contamination reduced.

MR. A. ROYCE, in reply, said that the sterile air was developed *in situ* by blowing the air into the screen and withdrawing an equal volume of mixed air and gas through a cotton-wool filter. The aim was to eliminate all organisms in the screen; therefore it was felt that the method used was better than using an aerosol. In the screw-capped containers satisfactory

growths were obtained with organisms in solid and fluid media. When the air space was of the order of a quarter or one-third there was no difficulty with aerobic organisms. He agreed that the word "almost" should be inserted. It was felt that the present method gave conditions as near as possible to absolute sterility, but it was agreed that under special conditions it could break down. If anything did go wrong one was left in no doubt that it had because of the gross contamination, but this had only happened twice in some 20,000 tests over three years.