The Art of Managing Contamination

By Jeffrey L. Tate

Use of the Safe-Septum® Prevents Microbial Contamination in Bioreactors

One of the major problems in the pharmaceutical industry, especially during long production runs, is keeping contaminant microorganisms out of the bioreactor. Contaminant bacteria, it seems, always multiply fast, produce undesirable byproducts and gases, and rapidly turn a clean production run into a nasty mess. Moreover, when rogue bacteria go on a rampage, you are usually faced with high cleanup costs, disrupted production schedules, and-most important-lost revenue.

This article describes a radically different type of septum-the Safe-Septum®. The Safe-Septum has been used successfully in regulated manufacturing facilities for more than 15 years to control microbial contamination. The Safe-Septum was subjected to a series of contamination tests during material transfers into and out of a series of small test bioreactors. The results suggest that the use of the Safe-Septum in the pharmaceutical industry would dramatically reduce the incidence of microbial contamination.

Anatomy of the Conventional Septa Versus the Safe-Septum

Conventional septa are flat, made of a pliable, rubber-like material, and are often supported on one or both sides by a flat metal screen. Because most bioreactors operate under a slight to moderate pressure, flat septa tend to bulge outwards in the sampling port.

The Safe-Septum on the other hand, is radically different than a conventional septum, as shown in Figure 1.

The Safe-Septum system consists of three parts:

• *Safe-Septum Boot*

The Safe-Septum, made of either silicone or EPDM, is non-coring, contains no metal screens, and provides a completely aseptic barrier for needle insertion and withdrawal. The Safe-Septum comes in two versions, a larger, twelve injection port version and a smaller, seven injection port version.

• *Safe-Septum Needle Guide*

This is made of plastic or nylon and contains a series of holes for guiding the needle through the Safe-Septum. The needle guide ensures that all needle insertions are completely separate and physically isolated from one another and, because each needle guide is covered on the outer side by a thin plastic membrane, clearly shows when any of the guides have been used. The Safe-Septum boot, needle guide, and plastic cover make up the Safe-Septum "cartridge," which comes in its own presterilized package ready for insertion into the Safe-Septum fitting.

• *Safe-Septum Fitting*

This fitting holds the Safe-Septum cartridge and is welded, threaded, or clamped in place in the wall of the bioreactor or process line. The fitting is capped by a stainless steel screw collar or hex nut, as shown in Figure 2, that firmly compresses the Safe-Septum into the sampling port.

The design of the conventional flat septum presents a number of drawbacks. First, contact with metal screens sometimes breaks needles during the material transfer process. Second, the lack of needle guides makes it difficult to identify previously used injection sites and when injection sites are reused, the risk of cross contamination is significantly increased. Third, flat septum is subjected to *tensive* forces. As a result, when a needle is inserted the hole often remains open, allowing the contents to leak out onto the surface of the septum where contaminant airborne microorganisms start to grow. Finally, flat septa

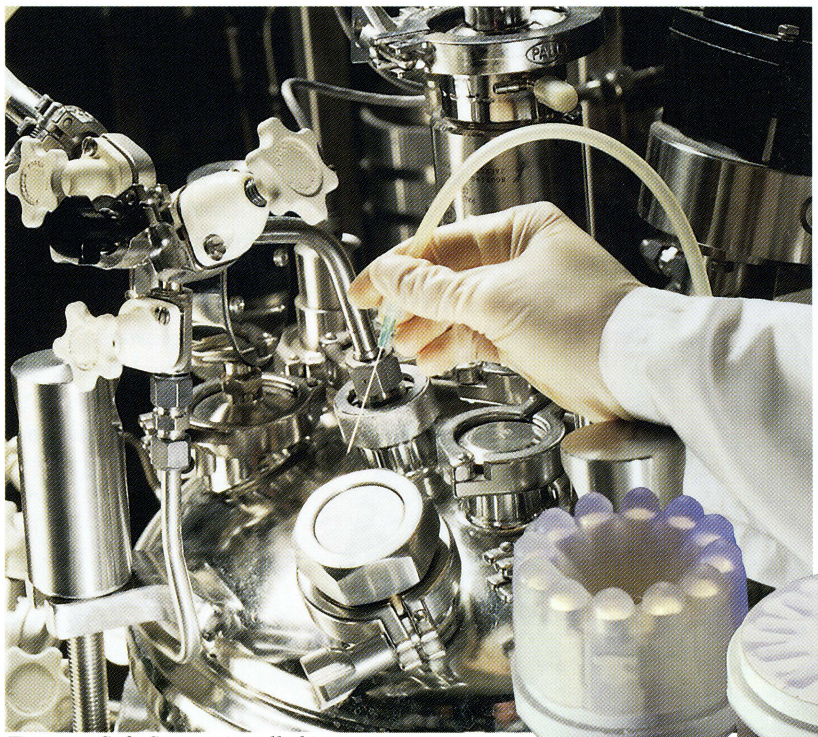

Figure 2. Safe-Septum installed in a bioreactor.

Figure 1. Seven- and twelve-port versions of the Safe-Septum.

sometimes rupture when exposed over long periods of time to high temperatures and pressures.

Unlike conventional septa, the Safe-Septum is subjected to *compressive* forces when properly fitted and provides an extremely tight seal when fitted to a bioreactor or process line. Because the Safe-Septum is held in place in the sampling port using a screw collar or hex nut, the Safe-Septum (and the walls of the Safe-Septum) are subjected to compressive forces that provide the motive power for hole closure when a needle is withdrawn from the Safe-Septum. As a result, the contents of the bioreactor do not leak onto the surface of the septum, airborne contaminants cannot grow on the surface, and subsequent material transfers are not contaminated by unwanted microorganisms.

To help technicians identify previously used injection sites, the exposed surface of the Safe-Septum is covered with a thin layer of white plastic so that used injection sites are clearly visible. When smaller diameter needles are used, the tip of the syringe can be pushed through the plastic layer to create a larger and more visible hole (Figure 3).

Validation Study to Test Safe-Septum Performance

In order to validate the performance of the Safe-Septum, it was challenged with high levels of bacteria to see how it performs under adverse conditions and to see whether it could be used to control contamination in the pharmaceutical industry-in particular during large-scale production runs. *Bacillus stearothermophilus,* a bacterium, was chosen as the test organism because it is widely accepted as an appropriate test organism for microbial contamination studies.

To determine how the Safe-Septum would perform in a regulated manufacturing environment, the Safe-Septum was studied under routine manufacturing conditions and was challenged by using *unrealistically* high concentrations of bacteria in a series of material transfer experiments.

Figure 3. Previous injection sites are clearly visible in the Safe-Septum.

Materials and Methods

The preparations for the experiments consisted of assembling six sterile bioreactors and preparing a culture of *B. stearothermophilus* to contaminate the material transfer process. In each treatment, the bioreactors were incubated for three days and tested for bacterial contamination as described in the "Incubation and Contamination Testing" section. In addition, each experiment was replicated three times.

Preparing the Bioreactors

Approximately 125mL (4.23 fl. oz.) of nutrient broth were added to six small cylindrical stainless steel bottles ("mini-bioreactors") with a capacity of approximately 1 L (33.8 fl. oz.). The mini-bioreactors were fitted with a stainless steel QMI fitting and a silicone seven-ported QMI Safe-Septum cartridge, and autoclaved for 35 minutes.

Preparing the Bacterial Culture

A storage culture of B. stearothermophilus (ATCC 12980) in a 40 percent solution of glycerol was removed from the freezer, streaked onto a nutrient agar plate, and incubated for several days at 60¡C (140¡F). When a vigorous bacterial lawn was visible to the naked eye, the plate was transferred to a sterile hood and the colony's surface swiped with a small sterile swab. The swab was transferred to a small sterile test tube containing 3mL (0.1 fl. oz.) of nutrient broth, capped, and shaken to distribute bacteria evenly throughout the swab.

Treatment One-Control

One of the mini-bioreactors was placed on a laboratory bench to simulate normal manufacturing conditions, and the exposed surface of the Safe-Septum swabbed for 20 seconds with a diluted solution of sodium hypochlorite to sanitize the injection surface. Five mL (0.2 fl. oz.) of sterile nutrient broth were drawn into a presterilized Monoject syringe fitted with an 18-gauge presterilized needle approximately 4 cm (1.5 in.) long. The needle was carefully inserted into the Safe-Septum and the nutrient broth injected into the bioreactor.

Treatment One was repeated as described above except that instead of *injecting* 5 mL (0.2 fl. oz.) of sterile nutrient broth into the bioreactor, 5 mL of broth were *withdrawn.*

Treatment One followed the standard operating procedures[1] established by the manufacturer (QMI) and served as the control for the contamination experiments.

Incubation and Contamination Testing

After nutrient broth was either added or withdrawn, each bioreactor was placed in a shaker-incubator at 60¡C (140¡F). After three days, the bioreactor was transferred to a sterile hood, opened, and a 1 mL (0.03 fl. oz.) sample of the nutrient broth withdrawn. The sample was transferred to a nutrient agar plate, streaked, and incubated at 60¡C (140¡F) for approximately 12 hours. The plate was then assessed to determine the presence or absence of bacteria. If present,

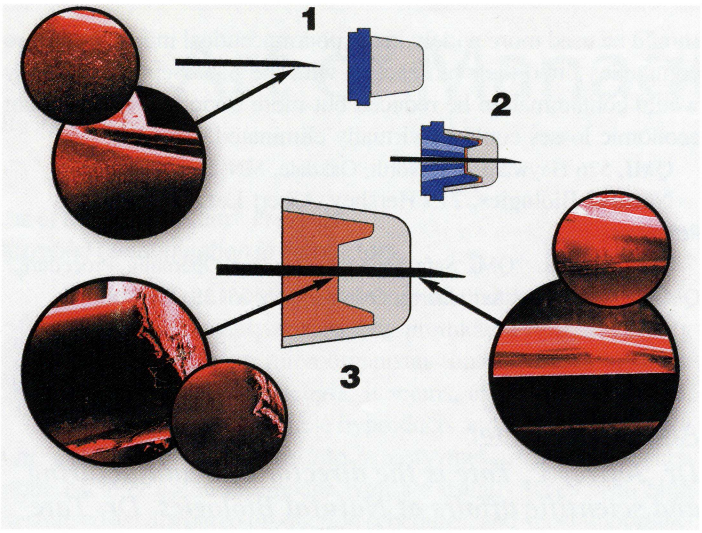

Figure 4. A contaminated needle emerges from the Safe-Septum free of bacteria.

the bacteria formed colonies that were clearly visible to the naked eye.

Treatment One, therefore, used all six mini-bioreactors in a single experiment. Consequently, once the experiments for Treatment One were completed, each bioreactor was disassembled, fitted with a new Safe-Septum, prepared as described above in the "Preparing the Bioreactors" section, and so made ready for Treatment Two.

Treatment Two-Needle Contamination

Using one of the mini-bioreactors, the exposed surface of the Safe-Septum was sanitized and a 5 mL (0.2 fl. oz.) sample of nutrient broth drawn into a syringe (as described in detail above under Treatment One). The external surface of the needle was then wiped down with a swab loaded with *B. stearothermophilus* to contaminate the needle. Care was taken not to let bacteria come into contact with the lumen (internal surface at the tip) of the needle. The needle was inserted into the Safe-Septum and the nutrient broth injected.

Treatment Two was repeated as described above except that instead of *injecting* 5 mL (0.2 fl. oz.) of sterile nutrient broth into the bioreactor, 5 mL of broth were *withdrawn*. Once these experiments were completed, each bioreactor was disassembled, fitted with new a Safe-Septum, prepared as described above in the "Preparing the Bioreactors" section, and so made ready for Treatment Three.

Treatment Three-Septum Contamination

Using one of the mini-bioreactors, the exposed surface of the Safe-Septum was sanitized and a 5 mL (0.2 fl. oz.) sample of nutrient broth drawn into a syringe (as described in detail above under Treatment One). The surface of the Safe-Septum was wiped down with a swab loaded with *B. stearothermophilus* to contaminate the injection surface. Using a sterile, *uncontaminated* needle, 5 mL (0.2 fl. oz.) of nutrient broth were injected into the bioreactor.

Treatment Three was repeated as described above except that instead of *injecting* 5 mL (0.2 fl. oz.) of sterile nutrient broth into the bioreactor, 5 mL of broth were *withdrawn*.

Scanning Electron Microscope Study

The purpose of this study was to observe in more detail what happens when a contaminated needle penetrates the Safe-Septum. In the open laboratory, a seven-ported silicone Safe-Septum cartridge was removed from its package, inserted into a stainless steel QMI fitting, and tightened into place. A syringe and needle were assembled and the barrel of the needle swabbed with *B. stearothermophilus*. The needle was aligned with one of the needle guides in the Safe-Septum and pushed all the way through until the tip and a reasonable portion of the needle barrel were visible on the other side of the septum. The syringe was disengaged from the needle, and the Safe-Septum transported to the scanning electron microscope (SEM) facility for imaging.

Results and Conclusions

The results of the experiments show that under normal operating conditions the Safe-Septum always safeguards the aseptic integrity of the bioreactor during the material transfer process. In addition, the Safe-Septum provides a significant margin of safety should either the needle or injection surface (or both) become contaminated during a material transfer. Moreover, the SEM study indicate the Safe-Septum controls bacterial contamination by physically excluding them from the injection site.

Contamination Test Results

The results of the contamination experiments show that when normal aseptic techniques are used, the Safe-Septum always prevented bacteria from entering the test bioreactors. Even when the needle and Safe-Septum were contaminated with unrealistically high concentrations of bacteria, the Safe-Septum prevented contamination from entering the bioreactors. Only when the needle lumen was contaminated did contamination within the bioreactor occur.

The results of our contamination experiments are summarized above in Table A.

TREATMENT				RESULTS		
	Septum	Needle	Broth	Replicate # 1	2	3
Treatment one	Sanitized	Sterile	Injected	S	S	S
(Control, SOP)	Sanitized	Sterile	Withdrawn	S	S	S
Treatment Two	Sanitized	Sterile, then C	Injected	S	S	S
	Sanitized	Sterile, then C	Withdrawn	S	S	S
Treatment Three	Sanitized, then C	Sterile	Injected	S	S	C
	Sanitized, then C	Sterile	Withdrawn	S	S	S

Key
S=Sterile
C=Contaminated

Table A. Results of the contamination Tests to Challenge the Safe-Septum.

SEM Study

Figure 4 summarizes the results of the SEM study, which looked at a needle entering and emerging from the Safe-Septum. The

scanning electron micrographs in Figure 4 clearly show that:

- the wall of the Safe-Septum fits very tightly around the barrel of the needle and excludes bacteria from the injection; (#2, Fig. 4) and
- after passing through the Safe-Septum, the tip of the needle emerges *completely* free of bacteria. (#3, Fig. 4)

It can be concluded, from looking at the scanning electron micrographs in Figure 4, that a properly assembled Safe-Septum system appears to "squeegee" contaminant bacterial from the surface of the needle. As a result, no microorganism can enter, and contaminate the bioreactor.

Significance of Our Findings

Contamination, unfortunately, is a fact of life in even the most carefully run research laboratory, pilot plant, or production facility. In my view, the art of managing contamination-especially in a major production setting-is to concentrate your efforts on those types of contamination that are most easily prevented. One of the easiest ways to prevent contamination is to use the Safe-Septum.

In conclusion, the Safe-Septum provides outstanding control of microbial contamination during the material transfer process and should be used more widely in the pharmaceutical industry. If more companies fitted their bioreactors with the Safe-Septum, not only would contamination be reduced but-more important-the resultant economic losses could be virtually eliminated.

QMI, 526 Hayward Ave. North, Oakdale, MN 55128.

Natural Biologics, 215 Hershey, Albert Lea, MN 56007.

References

[1] Anonymous. "QMI Safe-Septum® Standard Operating Procedure," QMI, 526 Hayward Ave. North, Oakdale, MN 55128, 1997. 4 pp.

About the Author

Dr. Jeffrey L. Tate is the director of manufacturing and scientific affairs at Natural Biologics. Dr. Tate conducted this study while he was the associate director of the Biological Process Technology Institute at the University of Minnesota. He has extensive research and consulting experience in the biotechnology and pharmaceutical industries.