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GEN CELEBRATES ITS 20TH ANNIVERSARY IN 2000!

## Bioprocess Tutorial

# Reducing Contamination of the Septum

### Controlling Entry of Bacteria Through The Sampling Port

Jeffrey Tate, Ph.D.

A major problem in the pharmaceutical industry, particularly during long production runs, is keeping contaminant microorganisms out of the bioreactor. A common entry point is through the sampling port, because conventional flat septa do not form a tight, aseptic seal. This not only provides microorganisms with an obvious point of entry into the bioreactor; leakage of the bioreactor's contents onto the surface of the septum encourages proliferation of airborne contaminants such as bacteria and fungi.

The Safe-Septum® can maintain the aseptic integrity of bioreactors—even under extremely contaminated conditions—by automatically resealing puncture holes, eliminating leakage and physically excluding bacteria from the injection site.

#### Flat Septa Prone to Contamination

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 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,

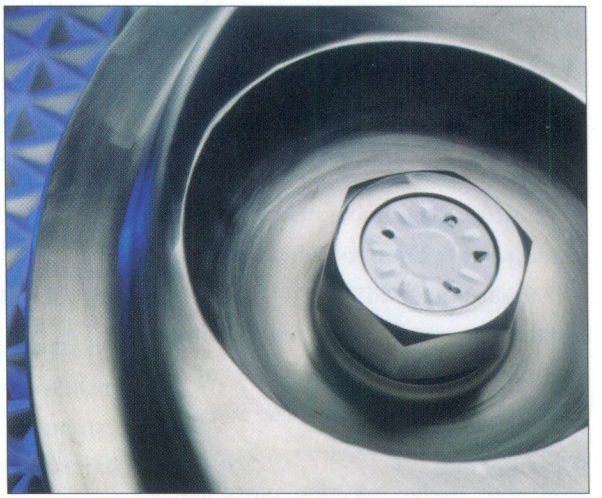

Figure 2. Previous injection sites are clearly visible.

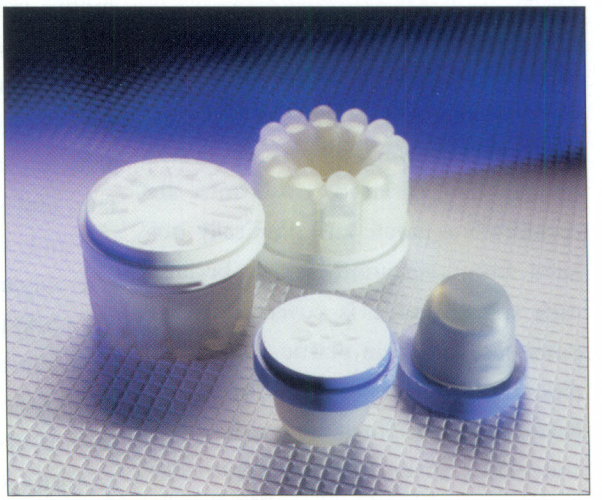

Figure 1. Seven- and twelve-port versions of the Safe-Septum.

because septa are subjected to pressure from below, the material composing the septum bulges outwards and is subjected to tensive forces, like the rubber in a tire or child's balloon.

As a result, when a needle is inserted into a conventional septum and removed, the hole often remains open. This allows the contents of the bioreactor to leak out onto the surface of the septum where air-borne contaminants such as bacteria, yeasts and molds start to grow. Eventually, the microorganisms either make their way into the bioreactor through the old injection hole, or contaminate a previously clean needle when a new injection site is used. Finally, flat septa sometimes rupture when exposed over long periods of time to high temperatures and pressures.

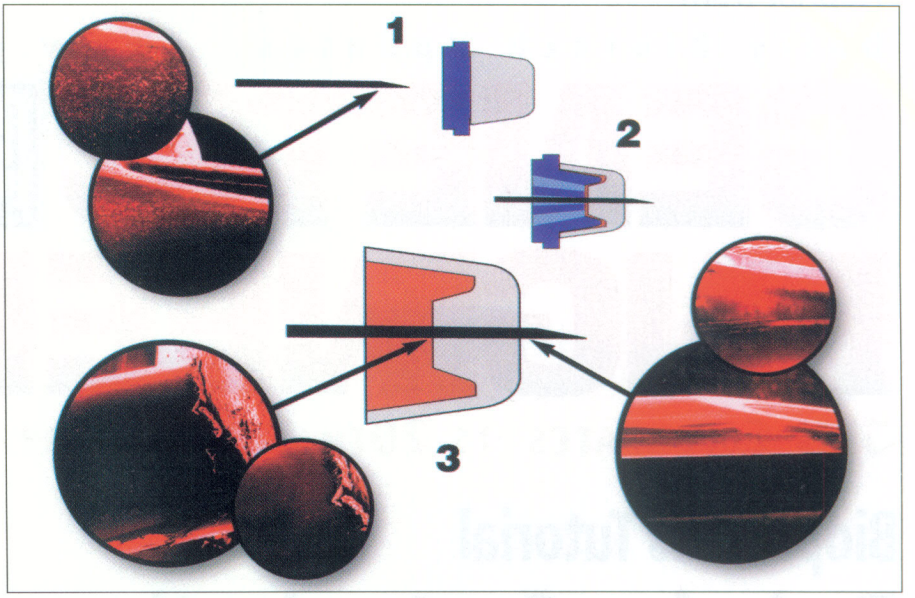

**Figure 3. SEM micrographs of a contaminated needle.**

### Safe-Septum Design

The Safe-Septum system consist of three parts: 1) The boot, which is 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 version with twelve injection ports and a smaller version with seven injection ports.

2) The Needle Guide, which 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 boot, needle guide and plastic cover make up a cartridge that comes in its own presterilized package ready for insertion into the fitting.

3) The Fitting, which holds the Safe-Septum cartridge, 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 that firmly compresses the Safe-Septum into the sampling port.

### Puncture Sites Reseal

Unlike conventional septa, a properly installed Safe-Septum provides an extremely tight seal when fitted to a bioreactor or process line. Thus, the Safe-Septum, and its walls, are subjected to compressive forces that provide the motive power for hole closure when a needle is withdrawn from it. 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. This design minimizes the chance that production personnel will use the same injection site twice. When smaller diameter needles are used, the tip of the syringe can be pushed through the plastic layer to create a larger and more easily visible hole *(Figure 2)*.

### Dairy Industry Roots

The Safe-Septum has been used successfully for more than 15 years to control microbial contamination and carry out aseptic material transfers in regulated manufacturing facilities. For example, prior to 1985, many companies in the dairy industry made yogurt by filling a large tank with milk, opening a hatch on top, and simply pouring in a culture of Lactobacillus and Streptococcus to start the yogurt-making process. Not surprisingly, many batches were lost to contamination.

Today, many manufacturers in the dairy, food-processing and brewing industries use closed bioreactors fitted with a Safe-Septum to control contamination. A recent lab study that replicated the closed, versus open conditions once favored by the industry, confirms that contamination caused by airborne bacteriophage is eliminated when bioreactors are fitted with the Safe-Septum. Its use in controlling contamination in dairy, food-

processing and brewing facilities led to its current use in the biotechnology industry.

## Validation Study

In a validation/contamination study, the Safe-Septum was challenged with high levels of bacteria to see how it performs under artificially contaminated conditions, and to find out whether it could be used to control contamination in the pharmaceutical industry—in particular during large-scale production runs. *Bacillus stearothermophilus* was chosen as the test organism as it is widely accepted as an appropriate test organism for microbial contamination studies.

The Safe-Septum was studied under routine manufacturing conditions and challenged by exposing it to unrealistically high concentrations of bacteria in a series of material transfer experiments. Both the outer surface of the needle and the outer surface of the Safe-Septum were deliberately swabbed with bacteria prior to needle insertion.

## Contamination Test Results

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 *(See Table 1)*.

Under routine operating conditions (where reasonable precautions are taken to ensure the cleanliness of the material transfer process), results show that the Safe-Septum always maintains the aseptic integrity of the bioreactor and prevents the introduction of microbial contaminants. In addition, the Safe-Septum provides a wide margin of safety should accidental contamination occur. Accidental contamination can occur when a technician omits to sanitize the septum, or forgets to wear sterile gloves and accidentally touches the needle while making a material transfer.

**Results of Contamination Tests to Challenge the Safe-Septum**

| | 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 |
| **Treatment Four** | Sanitized, then C | Sterile, then C* | Injected | C* | S | S |
| | Sanitized, then C | Sterile, then C | Withdrawn | S | S | S |

**Key:** S=sterile; C=contaminated; * needle's lumen accidentally contaminated with bacteria prior to injection

## SEM Study

The SEM study looked at a needle entering and emerging from the Safe-Septum. The scanning electron micrographs clearly show that: A) prior to entering the Safe-Septum, the tip of the needle is covered in bacteria (as expected, because the needle was deliberately contaminated in this experiment; *see Figure 3(1)*; B) the wall of the Safe-Septum fits very tightly around the barrel of the needle and excludes bacteria from the injection site (in fact, bacteria can be seen piled up against the outer surface; *Figure 3(2)*; and C) after passing through the Safe-Septum, the tip of the needle emerges free of bacteria (*see Figure 3(3)*).

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