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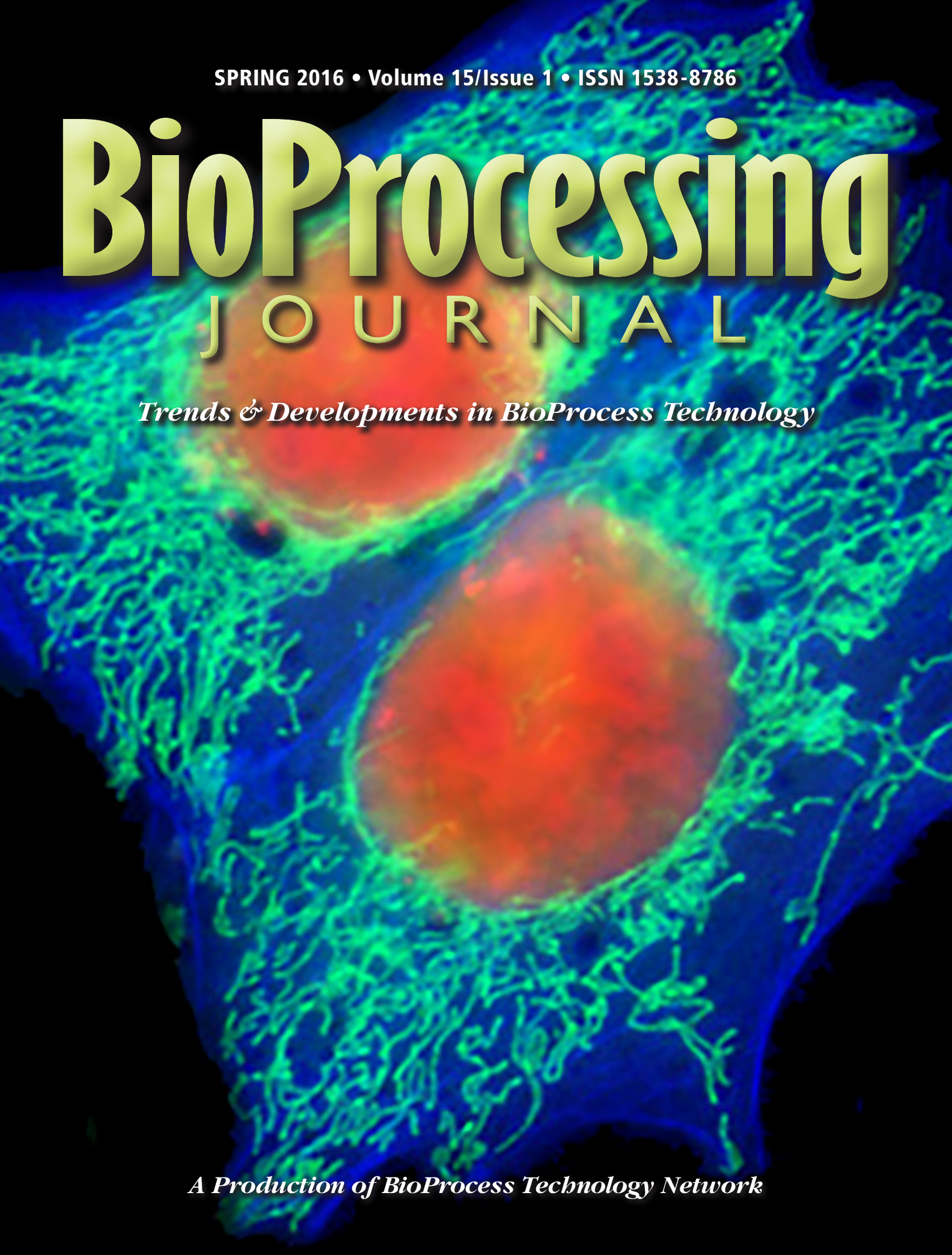

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TRENDS & DEVELOPMENTS IN BIOPROCESS TECHNOLOGY

The Contamination Triangle:

A New Model for Assessing the Risk of Biological Process Contamination and Performing Investigations

By Bill Ogden

Abstract

Microbial contamination is of great concern in pharmaceutical and biotech manufacturing. Many organizations struggle with determining the root cause of contamination when it occurs, and identifying effective safeguards to prevent future contaminations. This article will introduce a new model for understanding microbial contamination in biopharmaceutical and sterile products and processes.

The "Contamination Triangle" identifies the three factors necessary for microbial ingress into a sterile (or pure) system. The use of this model will provide guidance for contamination investigations, clarify the explanation of the contributing root causes in Non-Conformances, and assist in identifying risks and risk mitigation measures as part of a Failure Modes and Effects Analysis (FMEA) or other risk assessment method.

Introduction

Microbial contamination is of great concern in pharmaceutical and biotech manufacturing. Failure to prevent contamination may result in financial loss, product recalls, and regulatory action (including Warning Letters, Consent Decrees, product seizure or loss of license). Many organizations struggle with determining the root cause of contamination when it occurs, and identifying effective safeguards to prevent future contaminations. Microbial contamination is of particular concern in biopharmaceutical and sterile product manufacturing.

This article will introduce a new model for understanding microbial contamination in biopharmaceutical and sterile products and processes. The "fire triangle" is a model used by the fire safety industry, which states that three things must be present for fire to occur: fuel, oxygen, and heat. If any one of these is missing, there will be no fire.

The "Contamination Triangle" is patterned after the "Fire Triangle," and identifies the three factors necessary for microbial ingress into a sterile (or pure) system. The use of this model will provide guidance for contamination investigations, clarify the explanation of the contributing root causes in Non-Conformances, and assist in identifying risks and risk mitigation measures as part of a Failure Modes and Effects Analysis (FMEA) or other risk analysis method.

In principle, this model can be used to investigate any form of contamination resulting from the ingress of foreign material into any kind of process. This article will focus primarily on bacterial contamination of biological and sterile manufacturing processes.

It should be noted that this method explains how bacteria can enter a sterile, aseptic, or pure system. The failure to fully sterilize a system before use is a separate cause of contamination not covered by this model.

The Fire Triangle

Fire Triangle Example

The atmosphere contains approximately 21% oxygen and paper (a fuel) is combustible. Yet paper only burns if it is exposed to a significant heat source, such as a lighted match. It takes all three to have fire.

Once a fire is started, the oxidation of the fuel releases heat, which keeps the fire going until it runs out of fuel or oxygen. But fires can be put out by cooling them with water, smothering them with CO_2 (or another inert gas), or by taking away the fuel source. To prevent or put out a fire, you only have to eliminate one side of the fire triangle; any side will do.

The Contamination Triangle

The Contamination Triangle Explained

Three things are necessary to biologically contaminate a sterile or pure process: an

organism, an opening, and an operative force. So the three sides of the contamination triangle are:

- The **organism**—The presence of a viable microbial organism in the environment
- The **opening**—An opening into the process by which the organism may enter
- The **operative force**—A pressure differential or other force that moves the organism through the opening into the process

As in the fire triangle, all three conditions must be present in order to contaminate a sterile or pure process. Sterile filtration is based on this principle. Growth media may contain microbial contaminants, and the media may be forced through a filter with positive pressure, but as long as the membrane pore size is small enough and the filter is integral, there will be no opening for bacteria to pass through.

Fermentors and bioreactors can be operated without contamination even in outdoor environments surrounded by bacteria if the equipment is operated as a "closed" system. Aseptic filling and many other processes are open to the environment around them, so the environment is carefully controlled to exclude bacteria and the particulates that often harbor them.

Use of the Contamination Triangle in Contamination Investigations

Investigation teams often have difficulty identifying the root cause of a contamination. Sometimes they find it even harder to clearly explain the cause. The use of this model can help direct the investigation and clarify the write up.

Biological contaminations occur in one of two ways. Either one or more of the components were not sterile to start with (so the contaminating organism originated from within the system boundary) or an organism entered the sterile/pure system from outside the sterile boundary. While this model only addresses the second scenario, a thorough investigation should evaluate both possibilities.

When investigating the possibility of a contaminant entering the system from outside the sterile boundary, all three sides of the contamination triangle should be considered. When describing the root cause of a contamination, all three sides of the contamination triangle should be identified.

The Organism

The identification of the organism involved in the contamination has great value. In conducting criminal investigations, detectives consider the modus operandi (mode of operation) or MO of the criminal. Different types of organisms behave differently. One could say they have different MOs.

- **"Water bugs"** (usually gram negative rods) are commonly found in water systems or other wet locations, including condensate lines, drain lines, puddles in equipment, etc. Contaminations by "water bugs" are most often associated with leaks through boundary valves from process or drain piping.
- **"People bugs"** (often gram positive cocci like *staph, micrococcus,* and *strep*) are commonly found on the skin or mucous membranes of people. Contaminations by "people bugs" are usually associated with aseptic manipulations during open process steps.
- **"Spore formers"** (like *Bacillus*) are common environmental contaminants, but the spores are resistant to heat and disinfectants. *Bacillus* spores are the most common organisms involved in contaminations due to inadequate steam sterilization. Because they are so common in the environment, they may also be involved in contaminations of open processes or leaks into "closed" processes.
- **"Molds"** are commonly associated with wet environments (e.g.: water damaged dry wall). Unlike bacteria that reproduce by dividing, molds and other fungi can also release spores into the air like seeds from a dandelion. This can result in high airborne viable samples while the main colony remains hidden in a location that is not routinely cleaned.

Contamination investigations should identify the contaminating organism, where it is found in the manufacturing environment, and the most likely way(s) for it to get into the process.

The Opening

In order for an organism to contaminate your process or equipment, it must have a way in. Aseptic processes are routinely open, but even processes that are considered "closed" may be opened at times for some process steps. Common openings include:

- **Open processes**—Some process steps like filling vials, inoculum preparation in flasks, roller bottle operations, etc. are inherently open. These depend on clean environments and good aseptic technique to prevent microbial ingress.
- **Leaking valves**—There are a variety of reasons why a valve may leak, including improper assembly, loose bolts, diaphragm damage, an improperly set travel stop, etc. Sterile boundary valves are of particular concern.
- **Leaking seals**—Most process equipment (including disposables) contain a variety of seals, designed to maintain an integral boundary between the inside and outside environments. Seals may include:
  - Gaskets of various kinds (like tri-clamp or flange gaskets)
  - O-rings
  - Septums
  - Fitting connections (e.g.: hose barbs with tie-wrap)
  - Mechanical seals—Tank agitators and pump shafts often have mechanical seals.

- **Defective sterile tubing welds**
- **Filter integrity failure**—This may result from a defective filter or a leak path around the membrane. It should be noted that a single 1-micron defect could allow contaminants to pass, but most integrity tests would be too insensitive to detect this failure. Where sterility assurance is critical, adding a second filter in series would reduce the risk of this failure mode.
- **Compromising the sterile boundary by sequence of operations**—The opening of sterile boundaries must be carefully controlled. Ideally the outer environment will be sterile before opening it to the process. It is possible to control an opening between the sterile (or pure) system you need to protect and a non-sterile system, if positive flow from clean to unclean is maintained for the entire time the sterile boundary is open. It is important to never deadhead the flow path on the non-clean side while the sterile boundary is open. The sterile boundary valve should always be opened last and closed first.
- **Cracks in welds or other metal pressure boundaries**—While less common, leaks between the process and temperature control jackets can occur in heat exchangers and pressure vessels. Leaks can also occur at other locations between the process and the outside environment as a result of some kind of thermal or mechanical stress.

Contamination investigations should identify the opening through which the contaminating organism got into the process.

The Operative Force

In order for an organism to move through an opening and contaminate the process, there must be some kind of force to move it through the opening.

- **Positive pressure pushing it**—This force applies during the transfer or filtration of any liquid or gas into your process. This force often occurs at boundary valves when steaming or cleaning a connected system (e.g.: a transfer line).
  - **Overloading filters**—HEPA filters are generally rated as 99.99% efficient at filtering 0.3 micron particles. That implies that 0.01% may still make it through. Liquid filters are also validated for a particular microbial load. If the starting bioburden of the liquid to be filtered is higher than the filter rating, then some microbes may make it through the filter.
  - **Water hammer**—This is a short duration, high intensity sub-case of the positive pressure scenario. Slugs of water or other liquids can move through otherwise empty lines at high speed. The mass of a fast moving liquid will have a large momentum. The force of that momentum can transmit a high impact force on elbows, tees, valves, or other equipment where the flow of the liquid changes direction or is interrupted. This is most commonly experienced with condensate moving through steam lines. It is possible for cold condensate, CIP fluid, or some residual process fluid left in a line to apply water hammer to a sterile boundary valve at the initiation of steaming or CIP. This brief spike in pressure might cause momentary leakage of fluid. It only takes one droplet.
- **Negative pressure pulling it**—
  - As steam cools it condenses back into the liquid state. Since liquid water occupies much less space than steam, this condensation will result in a vacuum unless air, nitrogen, or some other gas is introduced to replace the steam and maintain positive pressure. The most common (unintentional) source of vacuums in biopharmaceutical and sterile processing occurs during cool down following equipment steaming. This is especially true of line SIPs where no source of sterile air or gas may be available.
  - The cooling of liquid or gas in a sealed container can result in a partial vacuum.
  - Some processes, like vacuum drying, intentionally pull a vacuum as part of the process.
- **Mechanical**—Mechanical devices that touch multiple things can transfer contamination from one to another. This is especially true of things like fill nozzles and pipettes that may be used to transfer liquids in or out of multiple containers.
- **Air flow**—Bacteria and mold spores can be carried along by air currents, particularly if they are attached to particulates. Turbulent air can move in very unpredictable ways. This is primarily a concern for aseptic processing, and is generally controlled through the use of HEPA filtration and unidirectional air flow. Even in controlled environments with unidirectional flow, anything that comes between the air source and the process opening can contribute contaminants to the air stream. When unidirectional air hits a surface (e.g.: hands, equipment, supplies, or the shoulders at the top of an open bottle) it will result in a localized area of turbulent flow.
- **Human hands**—It is commonly known that people are the largest source of contaminants in most cleanrooms. We shed skin flakes, cough, and sneeze. We carry environmental organisms on our shoes and clothes, and in our hair. Our hands come into close proximity to open processes, and our hands touch everything. We normally control this with sterile gowning and sterile gloves. However, it is easy to compromise the cleanliness of gloved hands by touching something contaminated.
- **Biological activity**—In some cases, the biological characteristics of an organism can contribute to moving it through the opening.
  - **Biological growth**—The doubling time of many bacteria can be as little as 20 minutes. As bacteria

multiply exponentially, the space they occupy increases as well.

- **Motile bacteria**—Many bacteria have flagella or other means of propulsion that enables them to move. Some are able to "swim" toward locations with higher nutrient concentrations (a process called chemotaxis). Many motile bacteria can swim at speeds on the order of 10–100 microns/sec. This is quite fast relative to their size, and equates to about 3.6–36 cm/hr. Bacteria do not normally maintain a straight path while swimming though. Even if they did, their speed would be too slow to overcome most pressure differential induced flow rates.
- While bacteria cannot swim against a strong current like a salmon, their motility and exponential growth could allow them to move through a stagnant or dead-headed line (such as a used sample line left full of media after use) or through a minute leak (such as slight seepage past a damaged O-ring).

Contamination investigations should identify the operational force that could have moved the contaminating organism through the opening into the process.

The Use of the Contamination Triangle in Risk Analysis and Mitigation

The Contamination Triangle model can be used to help evaluate the probability of contamination and identify ways to reduce that risk.

FMEAs, HAZOPs, and other methods are often used to analyze risks and identify ways to reduce those risks. Those methods usually analyze and score the risk in terms of severity, likelihood, and detectability. They then assign Risk Prioritization Numbers (RPN) by multiplying the three scores.

Biological contamination is generally considered to have high severity. Anything that can be done to identify and eliminate leaks before they cause contamination would lower the overall RPN by improving the detectability score. The Contamination Triangle model can be used to help evaluate and address the "Likelihood" component.

Calculating the Risk

In this model, there are three risk or probability factors: the likelihood of a viable organism being present, the risk of having an opening large enough for an organism to pass through, and the probability of having an operational force sufficient to move an organism through an opening. Since, all three risk factors must be present simultaneously; the probability of contamination for a given operation can be calculated by multiplying the probabilities of the three risk factors.

Another factor that can affect the probability of contamination by a given activity is how many times that activity is performed. If an activity is done more than once, then the frequency or number of occurrences must also be factored in. For example, the probability of contaminating a bioreactor during sampling might be estimated as once every 2,000 samples (0.05% per sample). If the bioreactor is sampled 20 times per lot, then the statistical probability of contamination occurring during sampling for any given lot would be 20 samples per lot × 0.05% per sample = 1% per lot.

In doing this calculation, the probabilities should be written as decimals (*e.g.:* 1% = 0.01). For example, if all three risks were estimated to be 10%, then the total risk would be 0.1 × 0.1 × 0.1 = 0.001 = 0.1%. This might be considered a fairly low risk if it is the probability per lot. If this is the probability per operation and that operation is done 50 times per lot, then the risk per lot would become 50 × 0.1% = 5%.

- **The organism risk**—This is the probability of an organism being present. For example, during the filtration of a non-sterile liquid, the probability of having an organism should be considered 100% (or 1) because the liquid is expected to contain microorganisms.
- The organism risk is often a subjective estimate of the probability. For example, if ungowned people are processing in an uncontrolled environment, the probability of an organism being present would be high, and might be estimated as 98% (or 0.98). This risk in a classified area would depend on the gowning and aseptic behavior of the people, and the ISO class rating of the location. It might be estimated as 25% (0.25) for operations with non-sterile gowning in an ISO-8 room. It might be rated as 0.1% (0.001) with good aseptic technique and sterile gowning in an ISO-5 hood. The risk/probability of an organism being present should never be considered zero.
- **The probability of an opening**—For open processes like filling and aseptic processing in hoods, the probability of an opening should be considered 100% (or 1). For a hermetically sealed vessel with no transfers in or out, the theoretical probability would be zero. Even equipment that is considered "closed" will have some finite probability of a leak depending on maintenance, operations, etc. Historical performance and observation may be the best way to estimate this. A probability of 1% (0.01) might be reasonable for nominally "closed" systems.
- **Operational force risk**—In some cases, the probability of an operational force that could move a contaminant through an opening could be 100%, as in a sterile vacuum drier. Bioreactors and sterile process vessels are often kept pressurized with sterile filtered gases to ensure any leakage is out, so the operational force risk would be low. In this case, it might be tempting to call the risk zero. Forces can be time, operation, and location dependent though. For example, there could be a positive pressure differential across a sterile boundary valve into a bioreactor during a line CIP or the steaming of an add port.

- **Frequency of occurrence**—This is the multiplier for how many times a specific operation is done during a lot. This could be 20 samples, 50 sterile tubing welds, 5 add port SIPs, etc.

Reducing the Risk

While minimizing each of the above risks might be desirable, the goal is to minimize the total risk. If the probability of any one of the three contamination factors can be reduced to zero then the total risk becomes zero. If none of the probabilities can be reduced to zero, then it would be best to minimize the probability of at least two of the risk factors.

Conclusion

This article introduced a new model for investigating biological contaminations and evaluating the risk of it occurring. The model is called the Contamination Triangle. It is similar to the Fire Triangle, in that three factors must all be present simultaneously in order for an organism to contaminate a sterile or pure process. The three sides of the Contamination Triangle are: the organism, the opening, and the operational force.

The likelihood of a contamination occurring during an operation is the product of the probabilities of each of the three risk factors multiplied by the number of occurrences for the operation being evaluated. The statistical nature of these risk factors and the resulting probabilities of contamination can lull people into a false sense of security when things are going well and baffle them when contamination does occur. So the next time you have to investigate a contamination, or perform a risk assessment related to contamination, remember the Contamination Triangle.

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