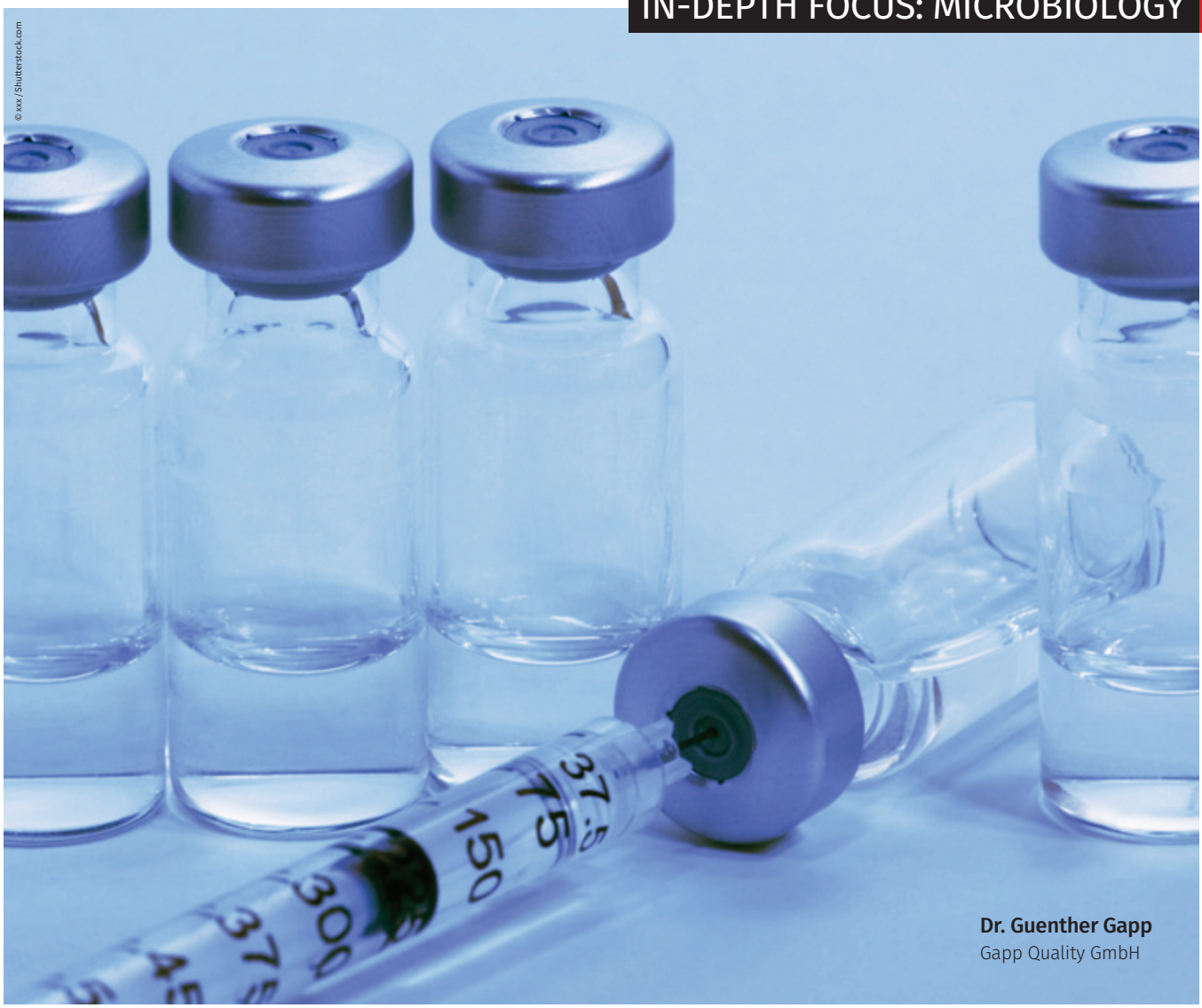


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# How to deal with non-sterile results in aseptic processing

**Non-sterile results in sterility testing (ST) and/or media fills (MFs) represent one of the most serious challenges for quality assurance (QA) microbiologists in the pharmaceutical industry. Investigation of the root cause, analysis of the risks to the product and determination of corrective and preventative actions (CAPA) take days, sometimes weeks, of manpower and resources. Decisions are taken under enormous pressure since they are always time-critical and have a huge impact on both the patient and the company. Therefore, it is essential to be well prepared in order to manage the investigation process proactively or, ideally, to prevent forthcoming non-sterile results.**

ST means a release test, where a small amount of a batch sample is tested whether it meets the specifications of the product in terms of 'sterility'. Because of test limitations, a passing ST result tells more or less nothing about the sterility of the whole batch. In contrast, a non-sterile result constitutes a strong alert signal that something has gone wrong. Since contamination is not usually equally distributed within a batch and the primary testing result is not reproducible in most cases, for example such as a chemical assay, repeated testing is not allowed without previous invalidation of the initial test.

A MF validation is a study carried out with the use of culture media

instead of the actual product. It is designed to evaluate and ensure the integrity of the whole process along with environment, operators, machinery and facilities being used for manufacturing sterile dosage forms. A non-sterile result in a MF validation may even be worse than a failed ST. On one hand it may indicate systematic failures in the plant or in the course of aseptic operations. On the other hand it has a higher impact: the batches produced in six months may be compromised, to the point of the very last successful MF. However, both require similar actions from all stakeholders involved.

## How to be better prepared

First of all, in contrast to terminally sterilised products, it is virtually impossible for any plant that produces aseptic products to prevent non-sterile results indefinitely. Rather, all participating parties are required to spare no efforts to develop and realise preventive action plans and to implement corporate culture in daily routine. In more than a decade of leadership experience and troubleshooting in the pharmaceutical industry the author has come to the conclusion that it is strongly recommended to strengthen teamwork, support multidisciplinary collaboration and to invest in training and commitment of the production personnel. Additionally, it may be helpful to devote particular attention to some specific topics in order to prevent forthcoming non-sterile outcomes, namely:

### *Focus: Bulk Material Quality*

A company fills aseptically produced 'sterile bulk material' from an external manufacturer into finished dosage forms (FDF), which may be syringes or vials. Usually, QA site managers have little impact on the choice of the supplier against economical or marketing aspects. In such a scenario, always be aware that low-priced sterile bulk material from an external supplier could imply to buy a black box! Even if supplier audits are performed, external two-day visits provide only limited information. However, filling a potentially contaminated bulk product renders all efforts of the best aseptic filling FDF plant useless.

Key message: Investments into in-house high quality sterile bulk production plants create indirect profitability by enhancing transparency and efficiency. In any case it will be worthwhile to invest in preventive maintenance of the complex manufacturing plant[1].

### *Focus: Facilities*

To minimise the risk of contamination it is most important to work with a modern, well controlled ST facility with isolator. In the old days (1990s and earlier) quality control (QC) microbiologists were in a difficult position to assure that their ST testing result had not been false positive. Nowadays most of the ST facilities are equipped with isolators, thus QC is in a much better position. Ideally this should be combined with a modern and well maintained production plant.

Key message: High grade equipment, such as a ST facility with isolator, may prevent false positive ST results.

### *Focus: Risk Management*

Structured quality risk management (QRM) and the proactive use of quality risk assessment (QRA) tools are both essential and required by the authorities to avoid non-sterile results. Several approaches are available, such as failure mode and effects analysis (FMEA), fault tree analysis (FTA) or hazard operability analysis (HAZOP). QRA tools should be used both proactively and periodically to assess the sterile/aseptic process. Three major advantages of QRA application/implementation include: 1) Early identification of weak points in the system allows initiation of remediation measures in advance; 2) Risk assessment constitutes a systematic process, where QA and QC microbiologists will review the manufacturing process together with other experts from production or engineering, thus operating together and strengthening teamwork; 3) Every involved party gets familiar with the process, the engineering systems and QC methods, prior to encountering deviations.

Key message: Proactive implementation of routine QRA procedures enhances security by identifying potential quality risks and promoting multidisciplinary collaboration.

### *Focus: Paperwork*

Be well-prepared for emerging non-sterility results by having good SOPs, flow charts and check lists for level 1 and level 2 investigations ready to hand on the very first day. Fishbone diagrams, Is/Is Not and fault trees may be useful tools for root cause investigation. As immediate actions are required, a targeted and systematic approach is indispensable for both, the first investigative steps and subsequent escalation management, field alerts or decisions on recall of the product.

Key message: In case of upcoming deviations, ensure that SOPs, checklists, investigative tools and related documents are ready to hand on the very first day.

### *Critical developments*

According to the author's experience, long-standing (> 10 years) highly-skilled employees are more important and helpful than good investigation tools. Unfortunately, especially in global companies, considerable staff turnover rates counteract those benefits. Additionally, the separation between QA and QC becomes more and more common in the pharmaceutical industry, so that involved persons often lose an overview of the process. In contrast, experts who know both sides are able to manage root cause investigations in a much more efficient way.

Key message: Try to retain as many skilled, experienced employees as long as possible within the company. Experienced long-term personnel and close cooperation of QA and QC experts strongly contribute to effective deviation management.

## How to deal with non-sterile results

As soon as an out-of-specification (OOS) result is detected in aseptic processing it is essential to react promptly and take immediate action, including:

1. Decision is required whether to STOP production or not, which may be a challenge in a campaign production. The situation is aggravated by the fact that the incident may date back up to 14 days, thus enhancing the possibility that important details may have been forgotten in the meantime.

Good maintenance of a sterile production plant is highly important to assure the sterility of the product

2. Daily meetings should be initiated in order to coordinate investigative measures and to encourage multidisciplinary cooperation. It is vitally important to refrain from mutual accusations (laboratory vs. production), to pursue solution-driven strategies and to operate as one team.
3. Ideally, the QA microbiologist should take the lead and keep track of all ongoing actions.
4. Usually, within a few days an escalation to global management and the authorities is required. Nowadays the global QA must be involved – which may be useful in certain situations. However, for site experts, such reporting obligation also implies some amount of additional work, thus binding capacities instead of dedicating more time to investigate the problem.
5. The use of rapid microbial testing methods facilitates immediate analyses of additional samples collected from the plant. Although the results obtained with those methods may be limited in terms of validity, they will provide valuable information within a few hours.

## Failure Investigation

In case of a non-sterile outcome in a ST or MF, the QA microbiologist is faced with the following five main challenges:

1. Find the root cause
2. Figure out how many batches are compromised
3. Make a correct release decision (for the patient's sake rather than to protect the interests of the company)
4. Set effective CAPAs
5. Write a detailed investigation report to keep record of all items and rationale of decisions, which is highly important for upcoming audits.

While contaminations detected during a MF validation usually affect only production itself, investigations after ST are extended over the following three areas:

A. Microlab (laboratory error)
B. Sampling (potential non-process error)
C. Production (process error).

## Microlab

Initially, an OOS result represents a deviation from the specified acceptance criteria. Therefore, the initial laboratory investigation is conducted in order to assess whether the sterility test procedure was carried out correctly. QC is obligated to check possible sources of error in the system, as there are e.g. negative controls, nutrient sterilisation, sample handling, the decontamination cycle of the isolator, environmental monitoring (EM) controls, bio-indicator exposures or glove integrity. Additionally, the investigation includes a review of qualification and training status of the QC personnel and a genetic identification of the contaminating germ. In some cases the classical metabolic profiles are very useful for cluster analysis and allow conclusions to be drawn about the potential origin of the contamination, such as e.g. a biofilm[2].

Common root causes in the microlab include:

- Contamination of the filtration manifold (backflow from the waste water)
- Negative control positive – e.g. enzyme inactivator (lactamase) has been contaminated
- Power failure in the isolator – continued testing against the requirements
- Propionibacterium strains are likely to survive in silicone oil of transfer devices.

## Sampling

Sampling means the collection of samples from the whole production batch. Usually, the sample is a worst case sample (e.g. pooled samples from the whole production charge for APIs). For FDFs, samples may be also collected after interventions, representing a worst case condition. A QA responsible person should be very familiar with the practical aspects of the sampling method and have the ability to assess its impact on a non-sterile test result.

## Production

When level 1 investigation does not yield that laboratory error caused the non-sterile testing result, a full-scale investigation (level 2) including a review of the production and sampling procedures should be conducted.

### *Part 1: Cleanrooms for aseptic processing*

Deficiencies in cleanrooms may lead to a non-sterile product, samples and/or MF failures during cleanroom operations. For example:

- No disinfection of material prior entering grade A
- Uncontrolled storage of sterilised equipment/packaging material in grade B
- Touch of product contact surfaces with gloves
- Performing risky interventions that have never been simulated in a MF.

Sterility of aseptically manufactured products requires best aseptic processing practices and a high commitment of production shop floor operators to quality, combined with sound QA systems. This requires motivation and appreciation of the shop floor personnel by their management. In his context an increased presence of QA microbiology personnel at the shop-floor level is also highly important, and beneficial for investigations. The role of a strong QA department to be 'on the floor' cannot be overemphasised in correcting and preventing MDDS from occurring. Avoid:

- Communication errors in connection with 'Lean Manufacturing Projects': shop floor employees including shift supervisors tend to believe that quantity of filled units is more important than quality.
- Cost saving programmes that result in understaffed production departments. The lack of cleanroom personnel requires fast working in cleanrooms, increasing the particle shedding of personnel and likelihood of mistakes.
- Understaffed QA departments as this compromises meaningful QA oversight in production.
- Inadequate salary, e.g. no incentive for cleanroom operators, although they work under inconvenient conditions and have a high responsibility.
- Pressure on cleanroom personnel not to run into EM deviations. As a result, incorrect execution of EM may lead to false negative results in cleanrooms with an additional lack of information. Since enforced by the industry, over recent years the situation has considerably deteriorated due to gradual tightening of EM limits within grade A (target = 0 colony forming units).
- Material transfer from 'wet rooms' into cleanrooms, combined with insufficient disinfection methods and ineffective material air lock.

### *Part 2: Production Facility*

Emphasising precision and perfection in maintenance procedures assures the integrity of the systems (e.g. piping, valves, gaskets and vessels) in order to prevent leakage, which may lead to contamination. The co-occurrence of leakage and interior vacuum (as used in vacuum driers, lyophilisators, centrifuges or storage vessels) may cause heavy microbial contamination by backsiphonage of drain water or environmental air. The latter also applies to inadequate transportation materials from external suppliers (leakage of cans and bags exposed to vacuum effects, subsequent to pressure fluctuations or temperature drops, especially during transport by air). Ineffective sterilising filters constitute another hazard source.

Clever ways for identifying root causes include:

- Rinse production plants with sterile liquids – make a segmentation and perform a microbial count testing of the rinse solution using rapid methods (very useful for investigation in closed systems)
- Open the plant, collect samples and perform swaps even from the surrounding non-sterile system. Try to recover the specific contamination strain.

Potential root causes associated with microbial contamination include:

- Sporeformers in the plant may originate from raw material (breakthrough) or from the drain system (together with gramnegatives)
- Growth of *Propionibacterium acnes* (microaerophilic) is often associated with carrier materials based on oil, e.g. emulsions. Protected by oil residues it may survive sterilisation in place (SIP) in consequence of bad housekeeping and cleaning procedures.

## Take home messages

If the root cause could not be found, never invent a root cause! Rather, reject the batch(es) and perform MFs before restarting production again.

Never lose sight of the enormous impact of a poorly performed investigation! The main focus has to be the patient.


**References**

1. Note: Companies who are able to sterilise bulk material by sterile filtration, are not affected by this special situation.
2. Note: Isolators are a lot less susceptible for testing errors. However, 'false positive' testing results may also occur with isolators.



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