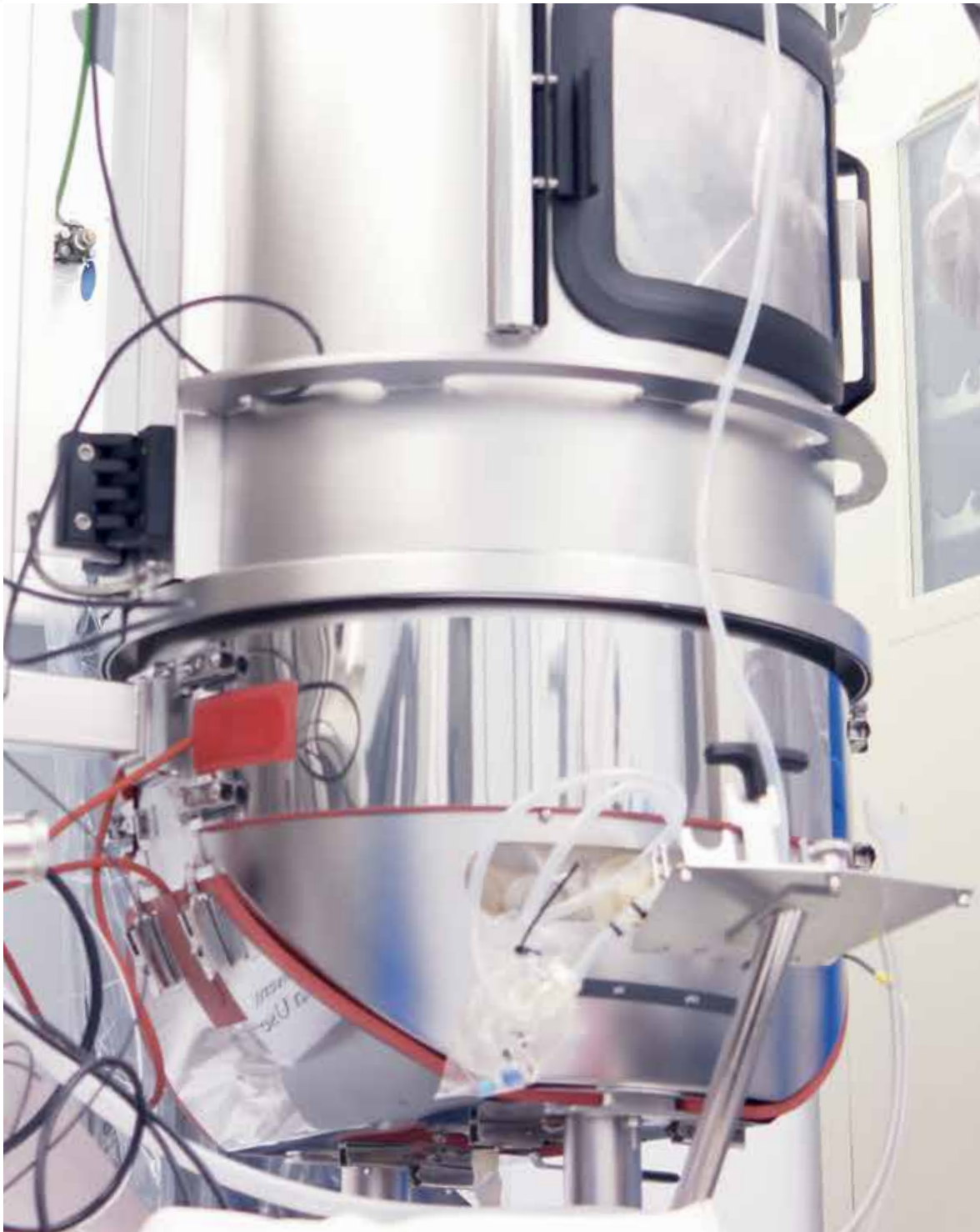
Updated GMO Containment Risk Evaluation Of Single-Use Bioreactors



CGM 2016-04

ONDERZOEKSRAPPORT

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Updated GMO Containment Risk Evaluation Of Single-Use Bioreactors

Client: COGEM
Date: June 10th, 2016
Document: 8247-REP-001 v4.0 FINAL

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The author would like to thank the following companies for their contribution to this report

- Uniqure
- Batavia Biosciences
- MSD Animal Health
- Halix
- Genmab
- Synthon
- Patheon
- Sartorius-Stedim
- Merck Millipore
- Thermo Fisher Scientific

The author would like to thank the following members of the steering committee for their contribution to this report:

- Danny Goovaerts
- Douwe Westra
- Nico Visser
- Bart Erkamp

This report was commissioned by COGEM. The content of this publication is the sole responsibility of the authors and does not necessarily reflect the views of COGEM.

Preface

The COGEM advises on a regular basis on the large scale manufacturing of GMOs in Single-Use Bioreactor (SUB) systems. These SUBs consist of a plastic disposable bag provided with all the necessary ports and mounted in a re-usable stainless steel vessel. The major advantage of these systems is their disposable nature, not requiring any cleaning sterilisation and validation after use. This increases the flexibility and capacity of a manufacturing plant considerably. In its assessment on the environmental risks of the use of these SUB systems, the COGEM also considers the findings of an evaluation report made by Xendo in 2010. This report describes several points of attention of manufacturing in SUBs and possible resulting risks like e.g. risk of leakage of these bags.

Over the past years the use of SUBs has increased considerably and this has resulted in an increase in knowledge and experience by manufacturers as well as end-users. Therefore, the COGEM commissioned a follow-up investigation into the current state of experience with a focus on the practical experiences with respect to the containment of GMOs. This investigation was also carried out by Xendo and consists of a literature study, a workshop with end-users and manufacturers, and where needed, individual interviews.

The authors conclude that aspects of integrity, leakage and improved bag design have received great attention and much progress has been made in better containing integrity risks. Short communication lines on all aspects of manufacturing in SUB's and intensive collaboration between SUB manufacturers and users have resulted in major improvements of several aspects of the manufacturing process, transport, installation, use and disposal of these bags. Adequate training in the use of these SUB systems was generally considered as one of the key elements and probably one of the most important factors to limit possible risks on leakage. Over the years, several procedures have been implemented to better control risks of leakage and it can be concluded that these risks are overall low.

The report describes in detail the most important factors that play a role in the different phases of the use of these SUB manufacturing systems and consequently this report can be a valuable aid for the risk assessment if COGEM is asked to give advice on the large scale use of these SUB manufacturing systems.

Danny Goovaerts

Chair of the supervising committee

Summary

The Commission on Genetic Modification (COGEM) has asked Xendo to investigate the current vendor and user experience concerning the use of Single Use Bioreactors (SUBs) in combination with Genetically Modified Organisms (GMOs). A special focus was placed on the construction and operational containment risks that were identified in the previous report written by Xendo in 2010. The Failure Mode and Effects Analysis (FMEA) that was part of the previous report has been recalibrated using the information collected during this investigation.

For this investigation, literature, users and vendors were consulted. Based on the information collected from these sources, it was concluded that the SUB technology has matured significantly over the last years. With increased experience at both the user and the vendor side, many improvements have been made that reduce risk for loss of containment by for example operator handling errors or SUB control system errors.

Over the last years SUB vendors have expanded their knowledge base concerning bag integrity considerably through close collaboration with users. SUB bag production processes are qualified. Shipping and transport occurs according to validated methods. Training is provided extensively to users of SUB systems at purchase and when significant design changes have been made. SUB production has improved over the years, yet between vendors there still is a different approach to SUB bag integrity assurance.

Users rely significantly on the expertise and quality assurance of the vendor for bag integrity. Most users do not employ dedicated integrity tests (e.g. pressure decay), but instead use a media fill test as their pre-inoculation bag test. Users also recognise that maintaining a high level of operator skill through (vendor) training and use of appropriate equipment is critical to successfully use SUB technology without containments events such a spillage or leakage occurring.

It was found that small defects located in the headspace of SUB bags pose the largest risk for loss of containment (especially when producing viruses which are transmissible through aerosols). These “pin holes” in the headspace are not readily found using a media fill test. A pressure decay test may not be able to indicate these holes as the pressure decay tests have a minimum detectable defect size based on bag volume.

Overall it is concluded that the use of SUB technology in combination with GMOs does not pose an increased containment risk compared to stainless steel vessels. This is based on:

- Any bag defect that could constitute a containment risk is typically spotted during the pre-inoculum phase.
- Operators are properly trained and use appropriate and validated equipment.
- Vendors have good control over their production process and raw material supply.
- Vendors and users freely and openly communicate experience and information and engage in continuous improvement of the technology.
- Between different types of SUB bag configurations, no significant differences have been observed.

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1 Introduction

The Netherlands Commission on Genetic Modification (COGEM) provides scientific advice to the Dutch government on the risks to human health and the environment with respect to the production and use of Genetically Modified Organisms (GMOs). The COGEM also informs the government on ethical and societal issues linked to genetic modification.

The biopharmaceutical industry uses GMO's like recombinant animal cells to produce recombinant therapeutics such as antibodies and vaccines for human and veterinary use. Since about halfway the previous decade the biopharmaceutical industry is increasingly adopting disposable, plastic culture vessels instead of the traditional stainless steel culture vessels to manufacture their products. Due to the relative fragile nature of these new, disposable culture vessels, this raised some concerns on the potential impact on GMO containment.
In 2010, therefore, an initial investigation [1] was commissioned by the Ministry of Health and Environment's Office for Genetically Modified Organisms (GMO Office) on the possible risks associated with the use of Single Use Bioreactors (SUBs) for the cultivation of GMO's. The resulting report, including a risk assessment based on the available insights at the time, has since been used as a reference document to support the licensing of new GMO related license applications.

Since the 2010 report, the use of SUBs for the cultivation of GMO's for both research and production purposes has increased significantly. This increase in use of SUBs has also resulted in a substantial increase in user and vendor experience and know-how, and has resulted in improved SUB design, manufacturing and operating procedures to mitigate and prevent loss of containment. Much knowledge has for instance been gained on integrity testing, handling related to leakage prevention and the general reliability of SUB operation.

Therefore the COGEM has commissioned a follow-up investigation into the current state of experience for SUBs. The results of this investigation have been documented in this report. This new report provide an update of the previous report based on the improved state-of-the-art in the field of GMO cultivations in SUBs and will re-calibrate the original risk assessments with respect to loss of containment in SUBs. Furthermore, some guidance is provided on practical measures to limit containment risks during the cultivation of GMOs in SUBs, which may lead to improved and streamlined permitting practice.

2 Scope and purpose of the study

The purpose of the study is to:

- Identify the user and vendor experiences with respect to containment of GMOs in SUBs within the BioPharmaceutical industry.
- Use vendor and user experience for the recalibration of the risk analysis which was performed for the GMO office by Xendo in 2010, to provide answers to the following COGEM questions:
    1. What is the user experience on the predictive value of integrity tests, the chance on loss of containment and the reliability of different bag types?
    2. How frequently does leakage occur and is this related to the different type of SUBs?
    3. Is it possible to develop an Standard Operating Procedure (SOP) based on these vendor and user experiences in order to minimise the "construction and operational containment risks" (for the most common SUB types available on the market)?

The scope of this study covers the cultivation of GMO's in single-use bioreactors with the following constraints:

- The volume of the liquid containing the GMO's is 10 litres or more.
- Single-use means that there are no "cleaning- and sterilization" cycles between re-use as in conventional, reusable stainless steel bioreactors, (decontamination before discharge is performed).
- Only those aspects that differ from conventional bioreactors will be discussed (except for additions to and samples taken from bioreactors).
- Only risks with respect to MI-I, MI-II and MI-III processes are in scope.

The following situations will be out-of-scope:

- Systems smaller than 10 liters.
- Non-bioreactors disposable bag's, like storage bags or recirculation bags (except for harvesting to such bags and transport of contaminated bags).
- Matters that are identical to conventional (non single-use) bioreactors.
- The impact on GMP.
- MI-IV.

3 Study approach

The following study approach has been used:

1. A literature study was performed to identify the current state-of-the-art in GMO cultivations in SUBs within the BioPharmaceutical industry. This literature was obtained from industry periodicals (e.g. BioPharm International), information provided by industry associations (e.g. BioProcess Systems Alliance (BPSA), International Society for Pharmaceutical Engineers (ISPE), Parenteral Drug Association (PDA) and other scientific sources.
2. A workshop was organised in which SUBs users from Dutch biotech industry shared their experience on the use of SUBs and the GMO containment “do’s and don’ts“ when using SUBs. In addition, individual interviews were organised for those who could not attend the workshop, and for Biotech industry members located abroad.
3. Interviews were held with manufacturers and vendors of SUB technology, in which the SUB production process and integrity assurance approaches were discussed.

4 Study results

The information collected during this study has been organized following each step in the life cycle of a SUB bag, going from Single Use Bioreactor raw materials to its production, shipment, installation, operation and disposal. Firstly, the previously identified theoretical risks in the 2010 COGEM report, have been briefly discussed. Subsequently the mitigating actions that have been implemented by Manufacturers and Users since the previous report have been described. Finally the effects of these mitigating actions on the occurrence and severity of containment failures has been discussed, followed by an update and recalibration of the FMEA table that was drafted in the 2010 report on containment risks when using SUBs.

The different phases described are

- Manufacturing of SUB bags
- Shipment
- Unpacking
- Installation
- Media fill
- Cell culture
- Virus production
- Harvesting
- Disposal

4.1 Manufacturing of SUB bags

SUB bag integrity control starts during production where the raw materials are combined into the final products. The main raw materials for SUB bags are the films that will be combined to form 2D or 3D bags. Furthermore, SUBs consist of ports or connectors welded into the film, internal inserts such as impellers and external additions, such as tubing, probe and tubing connectors, and filters. Assembly of the SUBs is still largely a manual process.
Besides SUB bags also ridged plastic bioreactors beyond 10 L scale are becoming increasingly available (e.g. Eppendorf BioBLU).
To ensure integrity after assembly, it is advised that vendors have a system in place to confirm bag integrity before shipment to the user.

4.1.1 Preventive actions from Literature

4.1.1.1 Integrity tests

In literature many different types of integrity testing are referred to, each having their own advantages and disadvantages. These tests are for example thermal imaging, pressure increase, pressure decay, helium integrity, vacuum bubble test and gas leakage testing. [2], [3]

Currently, in literature, there is still some debate on the specifications of critical defect size of SUBs. The critical defect size can also have different definitions e.g. 1/ as the size at which sterility is lost by microorganisms entering the SUB bag or, more relevant to this study, 2/ the size where GMOs can leave the SUB bag. The numbers mentioned range from 7 to 22 micrometre (µm). [3] This variation can be attributed to the differences in measuring methods and the microorganism used for the testing.
For any integrity test to be effective, it should be able to detect pin holes as small as the size of the critical defect size. This will depend on the accuracy of the integrity test, which in turn largely depends on the volume and type of SUB bag that is used.

In integrity test selection a trade-off needs to be made between several factors:

- Down time during testing
- Materials and equipment cost
- Impact of testing on bag and product
- Inherent flexible nature of bags
- Test sensitivity to leak size

Of the different integrity testing methods, the pressure decay test is one of the most commonly used within industry [4]. The ASTM provides a protocol for performing a pressure decay test [5].

This test method however has several disadvantages. A crucial factor influencing the effectivity of the pressure decay test is the size of the bag being analysed. In general it can be said that for larger bags, the accuracy of the testing method decreases. A pressure decay test for a bag of 200L is able to show defects with a combined size of up to 50 µm or larger. For a 2000L flexible bag this is approximately 600 µm. A pressure decay can also not indicate if there is a single or if there are multiple defects present. [2]

4.1.2 New mitigating measures by vendors

To mitigate the risks related to the production of the SUB bags, vendors have implemented the following measures:

4.1.2.1 Qualified procedures and work instructions for SUB bag production

Vendors have introduced SUB bag production lines which are qualified to ensure quality products. Part of the production process are the procedures and work instructions for the operators.

4.1.2.2 Integrity tests

All vendors involved in this study have a procedure for implementing some form of integrity testing during production of SUB bags. The approach however differed widely between vendors. The exact approach used is dependent on the point of view of the vendor.

One of the interviewed vendors performs a pressure decay test on all SUB bags produced.
Other vendors only perform integrity testing on the films before use in bag production. No integrity testing such as a pressure decay is performed as, according to these vendors, a bag cannot be reliably deflated in a reproducible manner.

All vendors perform a 100% visual inspection on produced bags. This inspection will look at all welds, seals, ports and other aspects. One vendor has developed a database of all known anomalies to them of which the impact on bag integrity and functionality has been established. During the visual inspection performed on their SUB bags, any anomaly found is compared to this database. When bag integrity or sterility cannot be guaranteed based on this analysis, the bag is rejected.

Despite these measures, pin hole integrity breaches may not be noticed.

4.1.2.3 Control of raw material supply chain

Vendors have realised that it is very important to control the supply chain of the films and materials used for the production of SUB bags. Over the recent years vendors have thus started to establish quality agreements with the suppliers of films and other raw materials. Changes or variation in quality of the materials can be addressed using these agreements.

4.1.3 New mitigating measures by users

To release SUBs for use in their facilities, users of SUB bags mainly rely on their vendors to ensure bag integrity and sterility. Common practice, and recommended as good practice, is that users audit their vendors to check and verify the quality systems in place. Aspects such as raw materials supply chain management, quality agreements, production procedures and work instructions related to loss of containment prevention are suggested to be part of these audits.
It is recommended that all relevant information is shared freely between vendors and users. [6], [7]

4.1.4 Re-calibrated risk

In recent years both vendors and users of SUB bags have taken steps to mitigate the risks on loss of bag integrity during production. The combination of increased control of the raw materials supply chain and quality agreements has led to a reduction in the numbers of rejected bags.

4.2 Shipment of bags

After production, the SUB bags will be shipped to the user. It is possible that the bags will cross various means of transportation and climates before arriving at the site of the users. Even then this does not mean that the bag will immediately be used for production. It is possible that the bag will remain in the warehouse for some time before the unpacking and installation takes place.

4.2.1 Previous risks

During transport many events can happen that might negatively impact the bag integrity or sterility. A non-exhaustive list of these events is given below:

- Vibration
- (Relative) humidity
- Temperature
- Handling
- Impact of objects

4.2.2 Mitigating measures by vendors

Vendors have realised that the users of their bags are located across the globe and have taken actions to mitigate risks related to this transport.
The container in which the SUB bags are transported have been optimized and specifically developed to withstand the extreme conditions that can be found in the different climates. An example of this would be the relative humidity of South-East Asia.
These containers have been validated according to internationally recognised standards from for example the International Safe Transit Association (ISTA) or the ASTM(formerly known as American Society for Testing and Materials).

4.2.3 Mitigating measures by users

For users of SUB bags, it is important to make sure that the warehousing and handling of the SUB bags is done according to the recommendations of the vendors. This can be done by for example proper environmental control of the warehouse.

4.2.4 Recalibrated risks

By improving the containers and packaging of the SUB bags combined with proper shipping and warehousing procedures and conditions, the introduction of damage to the bags during transport has been reduced.
Even so transport remains a very variable part of the SUB bag life cycle and every care should be taken to make sure that the bags maintain their integrity during the process.

4.3 Unpacking

4.3.1 Previous risks

It was found that use of sharp objects during the unpacking of bags could potentially result in a damaged bag. Also unpacking and unfolding of the bag can potentially introduce damage to the bag, the films or the seals and welds tubing and connectors that are part of the bag. This damage typically is caused by improper handling by operators.

4.3.2 Mitigating measures by vendors

During the redesign of the SUB bag transport containers to mitigate environmental effects on the bag, care was taken to adapt the container in such a way that damage during unpacking is minimised. Mechanisms to open the container without the use of sharp objects were introduced. Also special indicated regions where the internal packaging can be opened using a sharp object without damaging the bag were introduced.

4.3.3 Mitigating measures by users

The users of SUB bags implemented training programs for operators involved in SUB bag unpacking, installation and operation. These training programs are provided by vendors who provide these trainings on-site or at their own facilities.
Besides training, clear work instructions and procedures also assist in reducing defect bags caused by unpacking. Part of the unpacking is a visual inspection of the bag for any anomalies or defects such as white spots or stress marks.

4.3.4 Recalibrated risks

Unpacking and handling of the SUB bag will remain a risk as this involves human handling, but this risk can be mitigated through proper training of the operators, good work instructions and well-designed transport containers.

4.4 Installation

The chance to damage a SUB bag is the largest during the installation phase. After unpacking, the bag will need to be installed into the disposable bioreactor setup. Lifting the SUB, unfolding it, placing it in the holder, installation of stirrer shaft, connecting filters, inflation, installing probes and any other peripherals will take place during this phase. Due to the many different manual actions, the chance is realistic that damage to the SUB bag occurs.

4.4.1 Previous Risks

Installing the bag into the bag holder involves operator handling and a good understanding of the system. After the bag has been placed correctly into the system, it will be inflated.
The stirrer shaft will be installed during this part.

Any probes and sensors that did not come pre-installed will need to be installed in an aseptic manner at this point. These connections have been known to cause issues as leakage caused by either operator handling, damage during assembly or sterilisation.

4.4.2 Mitigating measures by vendors

Vendors have introduced several technical improvements to simplify handling especially for the large 500 – 2000 L SUB systems, such as lifting mechanisms, doors, collapsible or stackable impeller shafts, probe port support tools and more robust connectors.
Furthermore, the vendors have accumulated an intimate knowledge of their system over the past few years and experience in how to best install the different parts. This knowledge and experience is actively transferred to the users by providing up to date training and assistance.

4.4.3 Mitigating measures by users

4.4.3.1 Operator training

For users, certainly also from a commercial perspective, it is important to make sure that the operators working with SUBs are properly trained and experienced. Common practice, and part of GMP routine, is to train and qualify new operators for working with SUBs.
It is highly recommended that an effort is made to maintain an appropriate level of up-to-date knowledge and experience with respect to SUB bag related installation and handling, especially if a company is subject to a significant employee turnover. It is also important to have proper equipment and procedures in place for making tube connections, e.g. tube welding. It is recommended that this information is covered in work instructions and procedures.

4.4.3.2 Pre-use integrity test

During the user workshop some users indicated to perform a pressure decay test to test bag integrity. This is however not common practice as for several reasons:

- The pressure hold testing is inaccurate for large SUB bags.
  It was noted that the smallest total detectable hole size for bags of 2000L is 600 µm. For smaller bags, the resolution improves, allowing detection of smaller hole sizes. For example in a 200L bag the smallest hole reliably detectable as reported in literature is 100 µm [6]. But these pressure decay tests are not able to detect the minimum critical defect or whether there is a single or if there are multiple defects

- Performing a pressure decay test at the point-of-use of the bag (in the bag holder) is not considered to be very effective and not very operationally friendly.
  When the bag is in the holder, inflating it pushes the bag against the holder. This can effectively “plug” a hole. To prevent this plugging, one vendor has developed a solution in which a fleece jacket has to be installed between the bag and the bag holder [2], [8].
  However after performing the pressure decay test, this fleece jacket needs to be removed again. This introduces more handling of the bag, potentially increasing the risk of introducing defects. Currently there is little experience in the accuracy of the pressure decay test using this fleece jacket.

After installation the users will proceed with filling the SUB bag with medium. This will be described in section 4.5

4.4.3.3 Probes

Probes can either be pre-installed (as disposable probes) in the SUB bag or be re-usable conventional probes that need to be installed during bag installation. Pre-installed disposable probes remove extra operator actions and reduce loss of containment risks.
For the re-usable conventional probes, there are two critical steps where the risk on the loss of containment risk is high: installation and removal of the probes.

When re-usable probes need to be installed during bag installation, proper operator training and procedures as well as the proper support tools can minimise the risk of integrity loss. During vendor interviews it became clear that using single-use probes could potentially reduce the frequency of (small) loss of containment leading to contamination events by a factor 5 when compared to using conventional probes that need to be inserted afterwards.

After cultivation, probes can either be removed or remain in the bag for destruction. It is noted that some users use conventional probes in a single-use fashion during a single cultivation with subsequent destruction.

Installation and removal of re-usable probes pose a real risk with respect to bag integrity. If installed incorrectly (e.g. too much force, using damaged thread) leakage will occur, leading to loss of containment. The use of probes that are pre-installed removes much of this risk. If probes are used that require installation or removal, operator training remains the most critical factor contributing to risk mitigation.

4.4.4 Recalibrated risk

With an increased vendor involvement through training, maintaining a high knowledge and experience level of the operators and the use of the right tubing and equipment, the risks during the installation of SUB bags can be managed.
It became clear that using single-use probes can potentially lead to a significant reduction in (mostly small) loss of integrity events.
However, if there is any damage introduced during this phase, there is a significant chance that these will be detected during the next phase, the media fill, which is described in section 4.5.

4.5 Media fill

After installation of the SUB bag and its peripherals such as probes and stirrer shaft, the bag will be filled with the medium that will be used during production. The medium will be pumped into the SUB bag after which it will be heated to the required temperature before inoculation. Typically the bag will be filled with medium and kept overnight before the heating and inoculation take place.

4.5.1 Mitigating measures by vendors

To ensure proper filling and heating, vendors provide user support and operator training.
If any deviation such as a leakage may occur during the media fill, vendors have complaint procedures in which the incident is logged and root cause analysis support is given.

4.5.2 Mitigating measures by users

By performing the media hold test overnight, users will be able to spot most if not all leakages under the liquid level due to leaking seals or damage to the film caused prior to or during installation.

4.5.3 Recalibrated risk

The media fill is considered to be one of the most effective and operationally friendly integrity tests as it effectively demonstrates leakages and can be easily integrated into the routine process sequence.
Furthermore leakages are detected before the actual GMOs are entered into the system and therefore, although there is an integrity breach, this will not lead to a GMO spill.
It is therefore recommended to include the media fill and hold test as mandatory part of the installing and preparation of an SUB bag.

Main drawback of the media hold test is that defects above the liquid level can remain undetected.

4.6 Cell culture

4.6.1 Previous Risks

During fermentation the control systems of the bioreactor will provide control of in and out flows of the bioreactor. If these flows are not balanced, there is a risk that the SUB bag can rupture due to overpressure.
Other previously identified risks were defective temperature and stirrer control. An uncontrolled heating of the bag could potentially lead to the melting of the SUB bag films, causing a significant spillage. Breaking or disconnection of the stirrer shaft also has the potential of damaging the bag resulting in spillage. Also the use of harsh chemicals was seen as a potential risk for bag integrity.

Furthermore operator handling of the bioreactor during operation was identified as a risk for bag integrity. A SUB, its probes and in/outlets are connected with several tubes and cables to various other system parts. Movement around the bioreactor therefore could cause a spill, when an operator accidentally disconnects one of these lines due to tripping over it or bumping into it.
One example was heard of a pressure probe being disconnected due to such an event. This caused the control system to increase inflows as the probe indicated a low pressure. The SUB bag burst due to the subsequent increase in pressure.

4.6.2 Mitigating measures by vendors

Vendors have improved their SUB system design and improved many aspects such as control, interlock and stirrer mechanisms. Connecting lines and probes have been placed more securely, decreasing the risk of disconnection due to operator movement.
Other protection methods have also been introduced. For example several stage interlocks have been introduced to prevent the bag from rupturing due to pressure effects. When a pressure increase is detected, the control system can take several actions:

- Open exhaust valves (on in-flow or out-flow line) and generate an alarm
- Shut off gas flows into the reactor
- Shut off liquid flows into the reactor
- Stop process

Pressure protection similar to bursting disks used in stainless steel tanks have been introduced for some single-use bioreactors and serve a similar function. When the pressure exceeds a predetermined high level (alarm level) a controlled depressurisation of the vessel occurs without a significant spill. This mode of pressure protection is not commonly used in larger volume SUB bags.

Maximum temperature controls have been implemented to remove risks of the SUB bag melting. Stirrer design has also improved over the years. Easier installation and a more robust design has reduced occurrences of wrong installation with subsequent bag damage.

Improvements made to films over the years have increased the films resistance to chemicals that could potentially cause damage. It was concluded that the currently known feeds and bases used do not compromise bag integrity. As the bag comes into contact with the product, a extractable/leachable study will need to be performed. During this study typically harsher solvents and solutions are used compared to the conventional feeds and liquids used during the process and as such incompatible films do no proceed into production. It is advised to specifically log any impact on bag integrity during these type of studies by the suppliers.

4.6.3 Mitigating measures by users

During production in an SUB, operators will be performing many actions on the bioreactors. Making connections and taking samples were identified to be two of the activities with the largest potential risk for loss of containment.

Good operator training and the use of appropriate, validated equipment are the two main mitigation actions implemented by users

Extra care should be taken with respect to making sterile connections through for example tube welding.
However, this is also true for classical stainless steel bioreactors where the same tubing connectors are used.
During the user workshop, several observations were made that are crucial to minimise this risk.

- Use of proper and validated equipment (i.e. Tubing Welders)
- Training of operators
- The combination of material, tubing size, liquid and equipment needs to validated
- Monitor weld integrity over time
- Test impact presence of liquid in tube on welding performance
- Consider use of sterile connectors when tube welding fails still occur and remain high risk

Concerning temperature control it was noted that users should only use appropriate heating devices for the SUB bags as supplied by the vendor for the specific purpose. Usage of e.g. a heating device for a glass bioreactor on an SUB bag could potentially cause a bag rupture and spillage.

4.6.4 Recalibrated risk

Once an SUB has reached the stage of cell culture, very few issues are experienced with respect to loss of containment. Any defects or leaks in the SUB bag which are in contact with the media will be found as liquid will leave the bag. Any user who observes this will immediately stop production and perform cleaning to remove any spillage.

By improving the SUB system design, the control systems by for example interlocks and the films, vendors have made a large step in reducing any operational containment risks.
Users are recommended to maintain a properly trained operator pool that uses appropriate, qualified equipment.

When making sterile connections it is recommended that users take extra care.

4.7 Virus production

In terms of containment, virus production in SUBs was found to have additional risks when compared to for example monoclonal antibody production using mammalian cell lines. The main cause for this is the fact that virus particles are small compared to cells. It is also known that viruses can spread through aerosols. [9], [10]
During the user workshop discussions, it was observed that defects located in the headspace of the SUB will not be found through the media fill test. Performing a pressure decay test can indicate if there is a defect present, however is limited to the resolution of the testing device, bag size and method. Even though the size of these defects may be small (so-called pin hole defects), they do pose a risk in terms of containment.
Theoretically these defects can generate aerosols containing virus particles which then could contaminate the environment and pose a safety risk to operators.

4.7.1 Mitigating measures by vendors

As the interest in SUBs increase, vendors have started developing pressure decay devices with a better resolution, being able to detect smaller defect sizes.
Vendor interviews indicated that there have been no client request to counter pin hole defects in the headspace. One vendor did mention that recently they have seen an increase in users actively removing the SUB exhaust lines from the clean room environment. The exhaust was directly released

into the atmosphere in some cases, however the vendor noted that an incinerator could easily be introduced in the exhaust line to remove any risk for loss of containment.

4.7.2 Mitigating measures by users

During the user workshop it was observed that most users do not perform any additional integrity testing other than the media fill. One user mentioned that pinholes in the headspace part of the SUB could theoretically be detected by using a soap solution. Any defects would then generate bubbles. This solution was applied in a development setting, but was generally not considered practical for use in GMP settings.

4.7.3 Recalibrated risk

The presence of pin hole sized defects in the headspace of an SUB remains a risk for loss of containment on an SUB level.
It must be noted however that with increasing MI and BSL levels for more virulent products, additional containment precautions in terms of area classification and the gowning of personnel are necessary. The cleanroom in which the SUB is located is considered to be the next line of containment. Also operators will need to follow stricter procedures, for example required additional gowning and showering. These actions will reduce the risk of containment loss from a facility operations point of view.

4.8 Harvesting and Sampling

4.8.1 Previous Risks

Making connections remains a potential risk and it was noted that there is little difference in the risks here between stainless steel reactors and SUBs. For both types of bioreactors a wrongly executed action can compromise integrity and cause leakage.

4.8.2 Mitigating measures by vendors

Some vendors recognise that performing harvest and sampling is a risk bearing action. To mitigate these risks, some vendors have introduced sampling loops that are located outside the bioreactor. Introduction of this loop removes the performance of actions on the bag itself and thereby reducing risk.

4.8.3 Mitigating measures by users

For both the stainless steel and the single-use cases a proper operator training program is required to minimise construction and operational containment risks.
Also using the appropriate equipment is deemed as critical.

4.8.4 Recalibrated risk

By introducing design upgrade, implementing operator training programs and using appropriate equipment, the risks at harvest and sampling can be controlled. It is realised that any manual action performed on the system will bear risk and therefore remain a point of attention .

4.9 Disposal

After use of the SUB it will need to be decontaminated and its contents inactivated. Once this is completed probes and sensors can be removed from the bag. The bag can then be removed from the system and sent for disposal. When the SUB bags are transported off site, it will need to be transported in a suitable container as described by packing instructions 602 and 620 of the United Nations.

4.9.1 Mitigating measures by vendors

During this phase there is little effort required from the SUB vendors. If required, they could provide assistance in the development of the transport containers based on their SUB bag design.

4.9.2 Mitigating measures by users

For the users, removal of any peripherals from the SUB can still constitute a loss of containment. When a decontamination has been performed using a validated method for that organism, this risk can be mitigated.
Having procedures and work instructions describing all actions to be performed on the SUB and training the operators in these will reduce risk even further.

Transport of the SUB to the location processing (e.g. incineration plant) will have to be performed according the requirements set by legislation.
Packing Instructions 602 and 620 prescribe that genetic modified or infectious substances are transported in a three layered container.

1. The primary container should be leak proof
2. A secondary, leak proof container that should contain enough adsorbent material to absorb all liquids from the primary container.
3. A rigid third container that contains the secondary container and a listing of contents.

4.9.3 Recalibrated risk

By having proper operator training and procedures in place in combination with appropriate transport containers for used SUB bags, the risk of containment loss during the disposal phase can be reduced.

5 Overview Risks

In the previous report on SUB usage an FMEA has been performed to rate the identified risks. This was done by identifying the Severity (impact on containment), Occurrence (chance that it might happen) and the Detectability (is the loss of containment apparent). The Severity, Occurrence and Detectability are rated using a factor between 1 and 5. A Risk Priority Number (RPN) is obtained by multiplying these three factors. The RPN thus ranges between 1 and 125.

The Severity, Occurrence and Detectability factors as reported in the previous report[1] for the RPN calculation have been updated based on the current knowledge and experience. The new values are based on the discussion with users, vendors and other experts.

The FMEA has therefore been recalibrated based on current insights on loss of containment events including GMOs.
This means that the occurrence will be based on the chance that a loss of containment event has occurred after all mitigation actions were taken. For example an SUB bag which has been rejected during the media fill test will not result in a breach of containment and therefore have no impact on the occurrence.

The current update of this FMEA can be found in Attachment 1.

Severity (SEV)
1 = Hardly a loss of containment
2 = Miniscule loss of containment (droplets/ negligible aerosols)
3 = Minor loss of containment, small spillage (< 0.5-L / few aerosols)
4 = Loss of containment (< 10-L / aerosols)
5 = Severe loss of containment, large spillage (> 10-L / mist of aerosols)

No changes were made to the severity rating.

Occurrence (OCC)			Frequency
1	=	Very rarely heard of in industry	<<<1%
2	=	Rarely heard of in industry	<1%
3	=	Heard of in industry	<5%
4	=	Incidents have occasionally happened	<10%
5	=	Will happen regularly	>10%

The occurrence has been adapted to provide a more quantitative insight into the ratings.

Detectability (DET)
1 = Obvious and immediate (immediately noticeable, 24 hours a day, e.g. through automated control/alarms)
2 = Detectable through procedures
3 = Detectable with minor effort (noticeable by attention only)
4 = Detectable by active inspection (one has to actively search for the failure)
5 = Not detectable

The detectability rating has been adapted based on the progress of technology over time. The automation control and alarms is now included in rating level 1 as these react instantaneously when triggered.

This FMEA has been performed without any particular SUB or implementation in mind.
The recommended actions/fixes as reported in the previous report will not be repeated in this report. Risk assessment based on GMO class will also not be repeated in this report as Severity score has not changed significantly.

5.1.1 Outcome recalibration FMEA

The main impact of the advancement of knowledge, experience and technology over the recent years can be seen in a reduction of occurrence and heightened detectability.

Based on progressing experience, procedures and training programs have been developed to guarantee an efficient level of operator training (e.g. bag/probe installation) in SUB technology. This combined with improving technology (e.g. improved connector design, development of single use sensor technology) has reduced the occurrence of many issues present during the early years of SUB usage. Updated system configuration (e.g. probe protection, external sensor loops) has reduced the possibility for accidents that could jeopardise bag integrity.

SUB vendors have also updated their system based on feedback from users, integrating more relevant controls and alarms. Through training operators are now also more aware of possible risks and how to spot these (e.g. visual checking of bags for anomalies before and during installation). This has increased the detectability of potential failures.

The combined effect of a decreased occurrence with an increased detectability has decreased the RPN across the board.
It is noted however that SUB operation, like operation of a stainless steel bioreactor, involves human actions which continue to have an inherent risk.

6 Overview Best Practices

Based on the user workshop and SUB vendor interviews, the following best practises have been identified as important for minimisation of construction and operational containment risks.

1. **It is recommended to have adequate quality agreements in place between SUB vendor and user**
   Ideally the relationship between SUB vendor and user is open and transparent with clear communication between both parties. However, as the SUB vendor plays such a critical role, it is recommended that proper agreements are also in place. Topics such as service level agreements need to be clear for both parties and be agreed upon.
   It is advised that the SUB user performs a thorough audit of the SUB vendor to ensure quality standard are met. These audits are recommended to include quality systems that cover the raw material (e.g. film) supply chain of the SUB vendor and aspects such as complaint handling, change control, investigation support and operator training.

2. **Comprehensive operator training programs should be in place**
   Operator training is a critical factor. It is recommended that the vendor is pro-actively involved as they have deep understanding of their systems and can provide support when changes are made to for example the design of the system.
   For users it is advised to ensure a proper level of experience and level of knowledge of their employees. This is especially important if employee turnover is high.
   Ideally the training program covers all aspects that are part of SUB usage. The following non-exhaustive list are recommended to be part of the program:
   - Visual bag inspection before installation
   - SUB transport and installation
   - Probe installation and removal (if applicable)
   - Control and operation of SUB system
   - Sampling from SUB
   - SUB leakage and spillage
   - Any relevant integrity test that is employed by user
   - Any connection technology used (e.g. welding, sterile connectors (e.g. CPC))
   - SUB removal and transport of empty, used SUB

3. **Use of proper equipment**
   Proper equipment and procedures should be used for activities such as making of connections.

7 Conclusions

There has been a lot of progress over the last years in the field of disposable technology. Increased usage has led to better understanding and insight in the strengths and weaknesses of disposable technology. This resulted in an identification of the most important factors that contribute to minimisation of construction and operational containment risks.

It was observed that many factors that are important for SUBS are also critical for stainless steel bioreactors. Operator training and use of proper equipment are critical to ensure containment for both systems.

For SUBs, it is recommended to have proper quality agreements between user and vendor. Part of these agreements could cover the extent of bag integrity testing before shipment , availability of training for operators, support during deviation investigation and change notices concerning for example design changes.

The three research questions for this report and their respective conclusions are found below:

1. **What is the user experience on the predictive value of integrity tests, the chance on loss of containment and the reliability of different bag types?**
   The integrity test which is currently most commonly cited in the scientific literature is the pressure decay test. However the predictive value of this test is dependent on the volume of the SUB bag, with minimum defect size detectable increasing with SUB bag volume (600 µm at 2000L)

   SUB vendors use a different approach with respect to establishing SUB bag integrity. All vendors use validated production procedures to produce the SUB bag followed by a visual inspection.
   Several vendors do not perform an integrity test after production, stating that an integrity test is destructive. Performing this test requires the bag to be inflated and deflated, which introduces to much risk concerning bag integrity.
   One vendor performs a pressure decay test on all made SUB assemblies, stating that the deflating and shipping can be done reproducible and in a validated manner.

   The integrity test most commonly performed by SUB users is the media hold test, which is an inherent part of the production process (filling of the bioreactor with medium and the subsequent rise in temperature to bring the medium up to process temperature). Only 1 SUB user was found to use a pressure decay test at the point of use.

   The main contributors to the chance for loss on containment are the operators and equipment involved in SUB handling. The risk for loss of containment can be minimised by ensuring proper training of operators and use of suitable equipment,.

   Between bags and configurations, little difference was found in reliability.

2. **How frequent does leakage occur and is this related to the different type of SUBs?**
   Leakages that actually constitute an operational containment risk involving GMOs are very rarely heard off. In general, any defect that would cause a loss of containment will be identified during the pre-inoculum phase, after which the process will be terminated.
   It is noted that leakages could occur or become evident in the course of time.

3. **Is it possible to develop an Standard Operating Procedure (SOP) based on these vendor and user experiences in order to minimise the "construction and operational containment risks" (for the most common SUB types available on the market)?**
   Several critical factors contributing to construction and operational risk reduction in SUB usage have been identified in this report. Confirmation of the presence of SOPs covering these topics would contribute to the minimisation of construction and operational containment risks. These SOPs would cover the following topics:
   - SUB vendor qualification
   - Root cause investigation after contamination incident
   - Operator training on
     - Visual bag inspection before installation
     - SUB transport and installation
     - Probe installation and removal (if applicable)
     - Control and operation of SUB system
     - Sampling from SUB
     - SUB leakage and spillage
     - Any relevant integrity test that is employed by user
     - Any connection technology used (e.g. welding, sterile connectors (e.g. AseptiQuick-Sterile by Colder Product Company))
     - SUB removal and transport of empty, used SUB

8 List of Abbreviations

Abbreviation	Long text
BPSA	BioProcess Systems Alliance
COGEM	Commissie Genetische Modificatie Commission on Genetic Modification
DET	Detectability
FMEA	Failure Mode and Effects Analysis
GMO	Genetically modified organism
GMP	Good Manufacturing Practice
ISO	International Organization for Standardization
ISPE	International Society for Pharmaceutical Engineers
ISTA	International Safe Transport Transit Association
OCC	Occurrence
PDA	Parenteral Drug Association
RIVM	Rijks Instituut voor Volksgezondheid en Milieu National Institute for Public Health and the Environment
RPN	Risk Priority Number
SEV	Severity
SOP	Standard Operating Procedure
SUB	Single Use Bioreactor
µm	micrometer

9 Overview of Literature

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Attachment 1. Recalibrated FMEA for Single-Use Bioreactors

The FMEA found below contains 3 parts: the process input and potential failures (left section), the original FMEA (middle section) and the recalibrated FMEA (right section). The recalibrated FMEA also covers mitigations actions taken and any comments received during the user and vendor interviews.

Severity		Occurrence			Detectability	
1	Hardly a loss of containment	1	Very rarely heard of in industry	<<<1%	1	Obvious and immediate (immediately noticeable, 24 hours a day, e.g. through automated control/alarms)
2	Miniscule loss of containment (droplets/ negligible aerosols)	2	Rarely heard of in industry	<1%	2	Detectable through procedures
3	Minor loss of containment, small spillage (< 0.5-L / few aerosols)	3	Heard of in industry	<5%	3	Detectable with minor effort (noticeable by attention only)
4	Loss of containment (< 10-L / aerosols)	4	Incidents have occasionally happened	<10%	4	Detectable by active inspection (one has to actively search for the failure)
5	Severe loss of containment, large spillage (> 10-L / mist of aerosols)	5	Will happen regularly	>10%	5	Not detectable

Installation			**Original**							**Recalibrated**						
	Process Input	**Potential Failure**	**Potential Failure Effects**	**SEV**	**Potential Causes**	**OCC**	**Detectability**	**DET**	**RPN**	**Frequency reported by industry**	**SEV**	**OCC**	**DET**	**RPN**	**Actions taken**	**Comments**
1	Unfolding and placement of bags	Rupture of bags, seals, welds, tubing	Leakage, loss of containment	4	Wrong unfolding, improper attachment, misguided handling	2	Local puncture/rupture hardly noticeable	4	32	Very rarely heard of	4	1	2	8	- Training of operators, e.g. dry runs - Visual inspection during installation, reject when unaccepted - Integrity test before use	- Vendor-operator training improved significantly - Vendors more actively involved - Visual marks (e.g. wrinkling, white spots) may not be serious
2	Attaching stirrer shaft	Rupture bags/seals	Leakage, loss of containment	2	Improper attachment	2	Stirrer inside sleeve, impossible to see	5	20	Very rarely heard of	2	1	3	6	- Training operators - Medium hold test - Risk assessment if medium hold test cannot be performed	- Only for Hyclone - Chance bigger for big flexible bags - Some media do not allow for prolonged hold test - Higher risk (BSL) may require longer testing before running
3	Agitating uninflated bag	Bag rupture	Leakage, loss of containment	5	Operator or software failure	2	Noticeable during working hours	3	30	Very rarely heard of	5	1	2	10	- See immediately during installation	
4	Inflation of the bag	Rupture of bags, seals, welds, tubing	Leakage, loss of containment	4	Blockage exhaust, too high inlet pressure	3	Rupture of bag hardly noticeable	3	36	Very rarely heard of	4	1	2	8	- Pressure sensor interlocks - Relief valves - Filter heating to prevent condensation	- Leakage not always present at day 1
5	Installing conventional probes in the headspace	Probe holder damaged during autoclaving/ installed incorrectly	Leakage, loss of containment	2	Wrong sterilisation cycle/assembly	2	Leakage hardly noticeable	4	16	Very rarely heard of	2	1	2	4	- Operator training and procedures - Pressure hold test - Media hold testing	- Use a soap solution to detect bubbling due to leakage, bubbling means discarding
6	Installing conventional probes in the bottom	Probe holder damaged during autoclaving/ installed incorrectly	Leakage, loss of containment	4	Wrong sterilisation cycle/assembly	2	Leakage hardly noticeable	3	24	Very rarely heard of	4	1	2	8	- Operator training critical - Use single use probes - Media fill testing	- Some leakage does not occur immediately

Cultivation			Original							Recalibrated						
	Process Input	**Potential Failure**	**Potential Failure Effects**	**SEV**	**Potential Causes**	**OCC**	**Detectability**	**DET**	**RPN**	**Frequency reported by industry**	**SEV**	**OCC**	**DET**	**RPN**	**Actions taken**	**Comments**
7	Tube welding attachments	Defective weld	Leakage, loss of containment	5	Improper procedures	2	Wrong weld is detectable by operator	3	30	Rarely heard of	4	2	1	8	- Proper training - Proper equipment - Only use with appropriate tubing	- Use sterile connectors for larger tubes - Open tube parts can spring open, generating aerosols, in high MI/BSL levels this can be dangerous - Risks are dependent on product
8		Spillage	Spillage	2	Improper procedures or design	2	By design	3	12	Very rarely heard of	3	1	1	3	- Proper training - Proper equipment - Only use with appropriate tubing	- Liquid containing welds may spray once cut open between the clamps - risk during virus production
9		Leakage during cultivation	Leakage, loss of containment	5	Flaw of product quality/ misplacement of tubing	3	Noticeable during dayshift	3	45	Rarely heard of	4	2	1	8	- Proper training - Proper equipment - Only use with appropriate tubing	- Do not install welded part within peristaltic pump
10	Aeration	Bag burst due to blockage of exhaust filter	Spillage	5	Heavy foaming/ improper cooling/heating/ too high inlet pressure	3	Directly noticeable	3	45	Very rarely heard of	5	1	2	10	- Pressure interlocks, shutting off inlet gas and inlet liquids - Proper protection filters	
11	Headspace conventional probes	Leakage of probe seal	Spillage	2	Wrong assembly, malfunctioning	2	Leakage hardly noticeable	4	16	Very rarely heard of	2	1	2	4	- Proper training operators - Integrity test	For viral products, increased operator risk due to aerosol formation
12	Bottom conventional probes	Leakage of probe seal	Spillage	5	Wrong assembly, malfunctioning	2	Leakage is noticeable	3	30	Very rarely heard of	5	1	1	5	- Proper operator training - Use of disposable sensors - Media fill	Leakage is directly noticeable during media fill and production is terminated
13	Stirrer	Wear/ rupture of stirrer seal	Spillage inside stirrer sleeve	2	Wear	1	Stirrer inside sleeve, impossible to see	5	10	Very rarely heard of	2	1	3	6	-Terminate process when occurred	
14	Addition	Rupture of bioreactor or tubing due to overfilling	Spillage	5	Improper handling	2	Noticeable	3	30	Very rarely heard of	5	1	2	10	- Interlocks exist preventing overpressure occurring - Check for wear/tear of moving parts (e.g. pump tubing)	- Use proper connections(not e.g. tie-rips) - Check for "home-made" assemblies, proper use of materials

Cultivation and Removal			Original							Recalibrated						
	Process Input	**Potential Failure**	**Potential Failure Effects**	**SEV**	**Potential Causes**	**OCC**	**Detectability**	**DET**	**RPN**	**Frequency reported by industry**	**SEV**	**OCC**	**DET**	**RPN**	**Actions taken**	**Comments**
15	Temperature control	Melting biroeactor bag by improper heating	Spillage	5	Design flaw/ malfuncitoning temperature controller	2	Noticeable	3	30	Very rarely heard of	5	1	2	10	- Maximum heating tempature of heating blackets set to 44 degrees Celsius - Only use appropriate temperature controllers	- Only use proper dedicated equipment (e.g. do not use glass reactor heating jacket for plastic bioreactor)
16	Stirrer shaft	Rupture of bag due to breakage of stirrer shaft or bearing	Spillage	5	Mechanical flaw	1	Noticeable	3	15	Very rarely heard of	3	1	2	6	NA	Rarely heard of, stirrer shaft installation can pose issue in small rooms
17	Magnetic stirrer	Disconnection between magnet and stirrer resulting in overheating	Spillage	5	Mechanical flaw	1	Noticeable during working hours	3	15	Very rarely heard of	4	1	2	8	NA	Magnets used for this are very strong and do not easily disconnect
18	Removal conventional probes after decontamination	Open endings	Spillage	2	Improper procedures/ design flaws	5	By design	2	20	Very rarely heard of	2	1	2	4	- Proper training operators - Use conventional probes as single use resource	
19	Removal of bag from container	Rupture of empty bags/seals	Spillage	3	Improper handling	2	Hardly noticeable	4	24	Very rarely heard of	3	1	2	6	- Proper training operators - Immediately place bag in biowaste containers	
20	Transport of empty bag	Rupture of empty bag	Spillage	3	Improper handling	3	Hardly noticeable	4	36	Very rarely heard of	3	1	2	6	- Dedicated biowaste containers used - Vacuumize bag slightly before storing in biowaste container	- European / Dutch regulations concerning disposal/killing biological matter seen as strict
21	Transport of empty rigid bioreactor	Breakage of rigid bioreactor	Spillage	3	Improper handling	2	Noticeable	3	18	Very rarely heard of	3	1	2	6	- Dedicated biological waste transport containers used	Transport in container according to UN2814 - PI602
22	Chemical inactivation	Leakage due to incompatibility with inactivation agent	Spillage	4	Wrong chemical which is compatible with disposable bioreactor	1	Noticeable	4	16	Very rarely heard of	4	1	2	8	- Test chemicals in empty container before performing total production run	Leeachables/Extractables study should indicate ability of film/plastic to withstand harsh conditions

Additional			Original							Recalibrated						
	Process Input	Potential Failure	Potential Failure Effects	SEV	Potential Causes	OCC	Detectability	DET	RPN	Frequency reported by industry	SEV	OCC	DET	RPN	Actions taken	Comments
23	External risks - e.g. skid bumping	Damaging probes when bumping skid. Automation/ control malfunction causing: - temperature increase - pressure increase								Very rarely heard of	5	1	2	10	- Vendors installed protection around probes - Introduced tube racks - Install bumper rails around skids to prevent bumping