Best Practices on Media Fill

Best Practices Document

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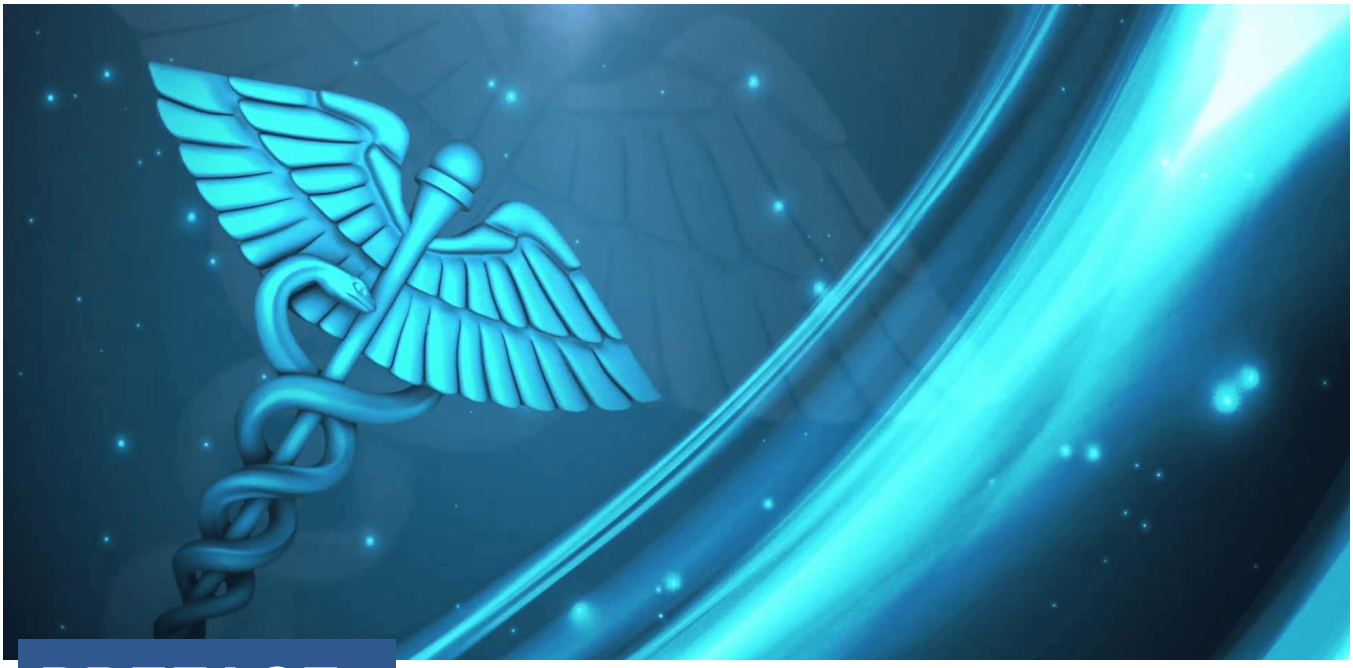


PREFACE

In April 2015, The IPA launched its Quality Forum (QF) to help Indian pharmaceutical manufacturers to achieve parity with global benchmarks in quality. The QF made a commitment to a multi-year journey to address key issues facing the industry and develop best practices.

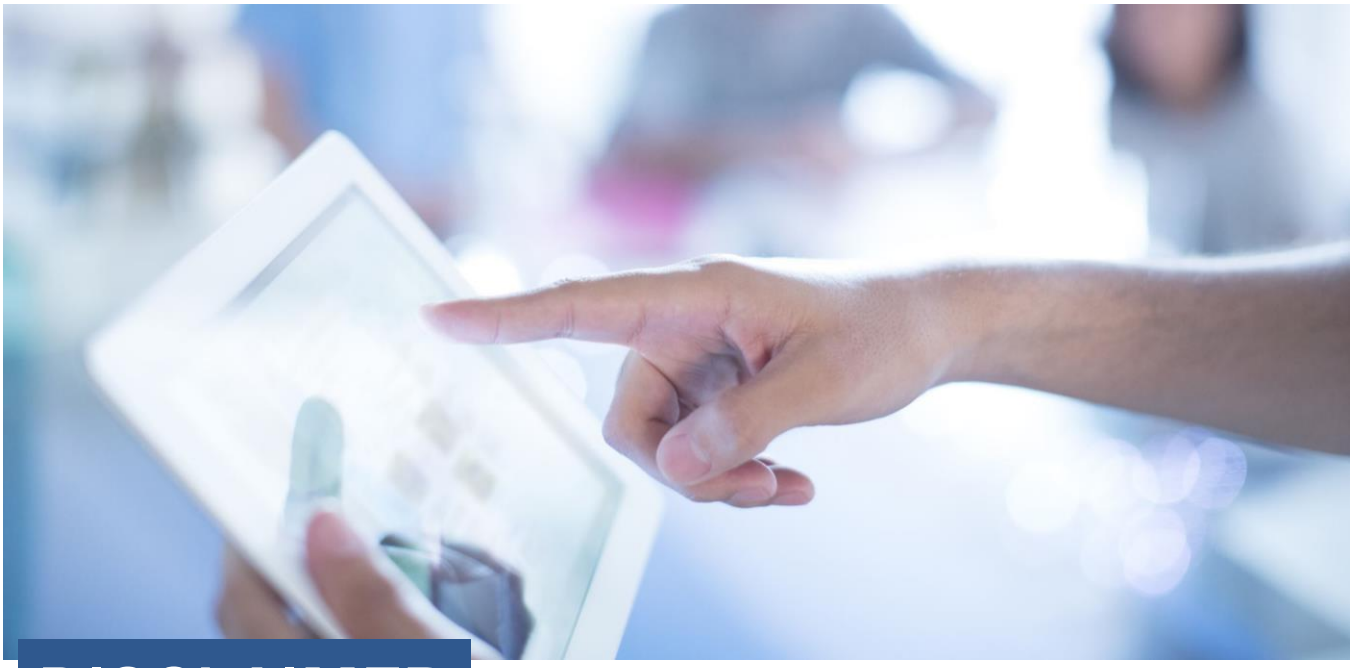
The QF focused on several priority areas in the last four years, namely, Data Reliability, Best Practices & Metrics, Culture & Capability, Investigations, etc. It took upon itself the challenge of developing a comprehensive set of Best Practices Documents for several of these topics. In this document, we focus on best practices for media fill.

The five participating companies in the QF nominated senior managers to study the best practices and frame the guidelines. They are: Davinder Singh (Sun Pharma); Pradipta Swain (Sun Pharma); Rahul Songire (Cadila Healthcare); Ashwin Upasane (Cipla); Sandeep Sharma (Cipla); Pradeep Moharana (Dr Reddy's); Kiran Deshmukh (Lupin). The IPA wishes to acknowledge their concerted effort over the last 12 months. They shared current practices, benchmarked these with the existing regulatory guidance from the USFDA and other regulatory bodies such as UKMHRA, WHO, etc., developed a robust draft document and got it vetted by a leading subject matter expert and regulatory agencies. The IPA acknowledges their hard work and commitment to quality.

The IPA also wishes to acknowledge the CEOs of five member-companies who have committed their personal time, human resources and provided funding for this initiative.

This document, to be released at the IPA's Advanced GMP Workshop 2021, will be hosted on the IPA website www.ipa-india.org to make it accessible to all manufacturers in India and abroad.

MUMBAI
OCTOBER 2021



DISCLAIMER

There are many guidance available for the Aseptic process simulation (Media fill). e.g. PDA technical report no. 22, EU Annexure I, Guidance for Industry from FDA, PICs and many more. Our little endeavor is to add into these from our industry experience, bringing better clarity to many situations we encounter and with risk based approach. This document also reflects the continuing changes and current requirements in media fill in view of patient safety and sterility assurance.

This guidance should be considered as a guide; it is not intended to establish any mandatory or implied standard. The reader must recognize that there may be additional requirements imposed because of new or localized and or any other regulatory expectations that are not included in this document. This chapter does not provide a universally appropriate template for the execution of process simulation studies. Each company must determine the appropriate rationale and approaches applicable to their relevant aseptic processes.

The use of risk assessments and related information may result in studies which go beyond the recommendations of regulatory authorities. If we focus on patient safety with risk based approach then it will automatically comply with all expectations. It may also result in studies which may slightly differ from various guidance. However, it should not result in studies which are less effective than those recommended by regulatory authorities.

1 Introduction

A. What is Media fill?

- Media fill [sometimes referred to as an Aseptic process simulation (APS)] is a validation to evaluate the sterility assurance / confidence of whole aseptic processing activities. However, aseptic product's sterility assurance can NOT be dependent on media fill only. Though not limited, it is dependent on all concerned personnel's training, experience, attitude, facility, process design, practices, understanding of risk and its mitigation etc.

- In media fill, a suitable microbiological growth medium is used in place of the drug solution during media fills to test whether the aseptic procedures are adequate to prevent contamination during actual aseptically manufactured / processed drug product/substances. This guidance is limited to drug products / formulations which are manufactured aseptically.

- Media fill studies include media preparation/compounding, filtration, filling, any other additional aseptic process. High risk aseptic operations should not be justified with successful media fill. e.g. transfer of open sterile contact parts/components in grade B without Grade A continuity, leakage post to sterile filter, power failure of Grade A air supply system etc. should not be justified by simulating in media fill.

B. Why media fill is required?

- Media fill (Aseptic process simulation-APS) is performed, as we can't evaluate or test each and every units for sterility of aseptically filled products. Sterility test is performed using representative samples and not on entire products as it is destructive. Despite of best efforts and technology, aseptic process possess some degree of risk during its processing. Hence, in a batch a single/few/some contaminated unit may remain un-detected if the same is not part of the sterility test sample.

- So APS is performed to identifying potential risk areas in an aseptic process that might contribute to the microbiological contamination of the product during processing. It evaluates the aseptic assembly and operation of the critical (sterile) equipment, qualify the operators and assess their technique, and demonstrate that the environmental controls are adequate to meet the basic requirements necessary to produce a sterile drug products by aseptic processing. The media fill does not validate the ability of the filter to sterilize growth media.

- The aseptic process simulation also provides evaluation of changes made to an aseptic processing operation which might impact the sterility of the final product.

C. Applicability and whether media fill is required for terminally sterilized (TS) product or not?

- Media fill is not required for terminally sterilized (TS) product(s). Even when TS product shares the same filling line with other aseptic product, the media fill for TS product is not required. Media fill is required for aseptic products processed on a particular line/area to produce the aseptically manufactured and/or filled finished dosage forms (human and veterinary) as well as manufacturers of sterile labelled bulk drug substances (active pharmaceutical ingredients).

- Media fill is not applicable for the drug product/ substances and/ or the processing lines, involved to produce terminally sterilised(TS) product/ substances and non-sterile product.

- However, if there is a combination of aseptic process and heat treatment for bio-load reduction (not terminal sterilization), then media fill is required.

- Note: If aseptically manufactured product and terminally sterilized product shares the same filling line, care to be taken to ensure that the aseptic processing line is not contaminated due to any kind of non-sterile component and/or non-sterile/low bio-load bulk handling for TS product. In such case, it is always necessary to manufacture the TS product with all care and sterilizations needed as if it is an aseptic product though media fill is not required for TS product.

D. Starting point and end point of media fill simulation

- Media fill simulation of the aseptic process starts from “the point of product and component sterilization to final closure/sealing of the primary container” (including any process/ handling steps subsequent to sealing that might impact container integrity). e.g. from sterile bulk manufacturing, sterile API addition (if any), sterile filtration, sterilization of components, set up of line, sterile sampling (if any), aseptic interventions, filling till capping of the vials, sealing of ampoules, post the stoppering of PFS etc. If there is a leak testing, post to the sealing/capping/stoppering of product, then the same (leak testing) to be included in the media fill process simulation.

E. Different synonyms of media fill

- Media fill synonyms are Aseptic process simulation, process simulations, simulated product fills, broth trials, broth fills, media fill Test (MFT).

2 Useful terminologies and definitions

- **Intervention** : An aseptic manipulation or activity performed by personnel that occurs within the Grade A zone.

- **Intervention, Corrective (Non-routine intervention)** : An intervention that is performed to correct or adjust an aseptic process during its execution. Examples include such activities as: clearing component misfeed/toppled/jammed on track, adjusting sensors, and re- placing equipment components (if any) etc.

- **Intervention, Inherent (routine intervention)** : An intervention that is an integral part of the aseptic process and is required for set-up or routine operation and/or monitoring, e.g., aseptic assembly, container replenishment, environmental sampling, etc. Inherent interventions are required by batch record, procedure, or work instruction for the proper conduct of the aseptic process.

- **Operator Fatigue** : The condition in which operator can work in a single stretch to operate the same filling line and/or aseptic processing. It is a state (physiological or psychological) characterized by some level of exhausted capacity to perform the task, and usually accompanied by feeling of tiredness and it is the most challenging condition for the aseptic processing.

- **Population-1** : The media fill containers which are Integral Containers (non-leaking and no cracks) that represent the same as that would be forwarded for packaging or considered for the use. This population also includes cosmetic defects from visual inspection or sorting process (the container should be intact enough that there is no chance for the breaching of container closure integrity). Any contamination unit found in population 1 is considered as media fill unsuccessful.

- **Population-2** : Those containers that would be discarded following the specific discard requirements for type of aseptic interventions and associated clearance specified in commercial production run as per manufacturing process instructions. However these containers should not be discarded in media fill. These are to be incubated as population-2 to assess actual risk to the practices during media fill. For purposes of media fill, this population of units with closure, capping and sealing and will be incubated separately with conspicuously labelled as population -2.

- E.g. If a production procedure requires removal of specific number of units to be rejected after an intervention like removal of 12 containers upon initial set up, vibrator bowl/ chute adjustment, batch records for production and media fills should clearly document that in media fill, that these should not be discarded. However the same 12 container to be sealed/capped and kept separately in population 2 with appropriate status labelling. Any contamination unit found in population 2 is to be recorded and to be investigated. Impact assessment to be done though based on successful result of population 1, media fill can be considered successful.

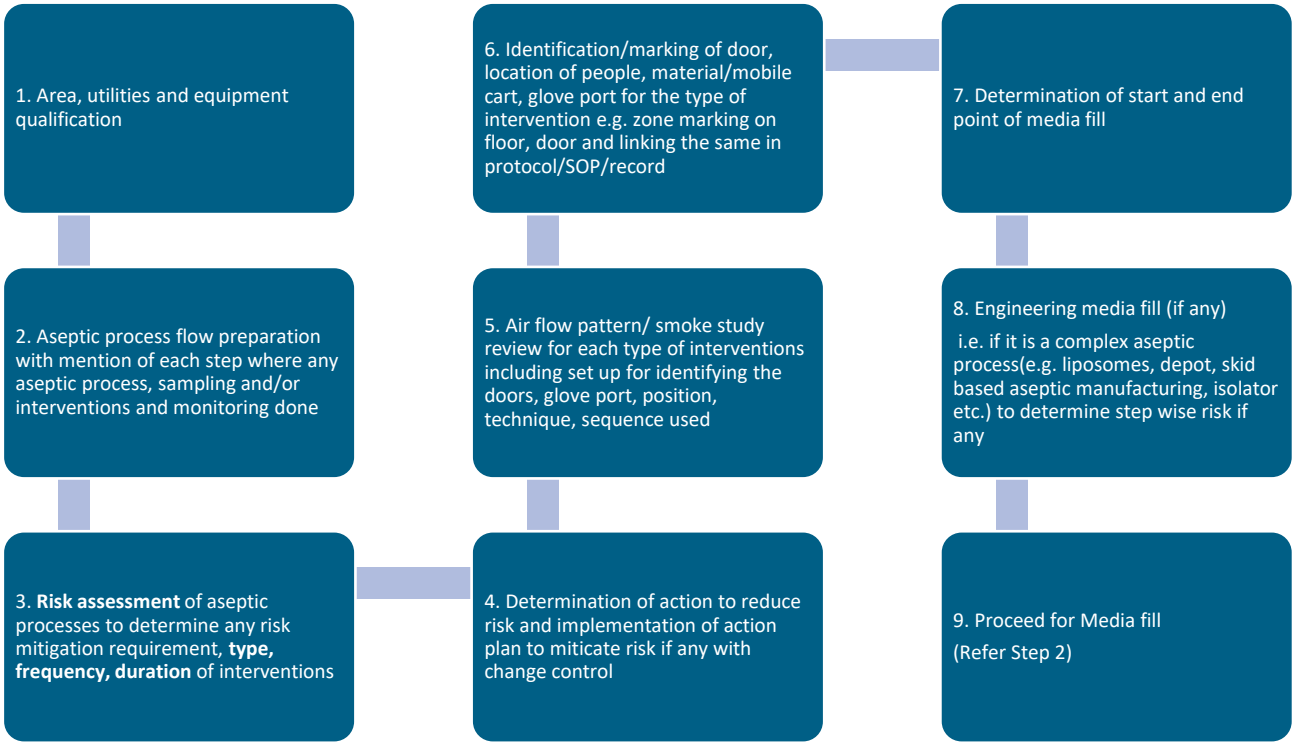
- **Worst Cases** : A set of conditions encompassing critical process steps and circumstances which as such conditions do not necessarily induce product or process failure. However this should not be treated in regular practice. If an inadvertent situation lead to worst case, then one of the impact assessment can be done by assessing outcome of such worst case scenarios in media fill i.e. any other risks also to be assessed. Worst case should not be granted as a routine practice.

- **Intrinsic failure** : A failure to meet qualification or validation acceptance criteria resulting from process failures.

- **Extrinsic failure** : A failure to meet qualification or validation acceptance criteria resulting from non-process occurrences. E.g. power failures or equipment failures.

3 Process flow of Media fill (APS)

Step 1: Pre-requisites for media fill



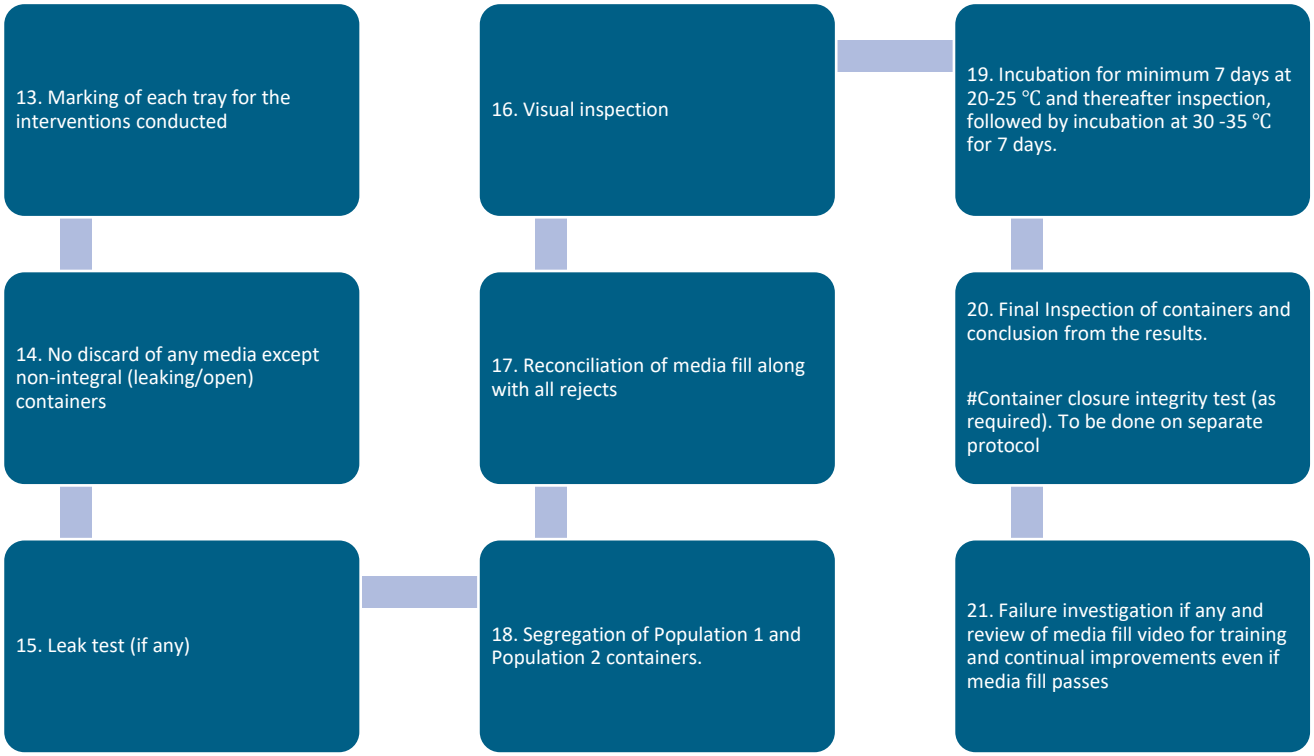
3 Process flow of Media fill (APS)

Step 2: Media fill* (Example given below is in reference to simple liquid container filling)

*This process flow may change from Step 7 onward depending on each aseptic process design for which media fill is to be performed.

3 Process flow of Media fill (APS)

Step 3: Identification, Incubation and inference (continued from above)



#Container closure integrity test (CCIT) is to be performed post to 14 days incubation on successful media fill batches.

Note: CCIT is an independent action for establishing container and closure integrity from microbial challenge prospective under both pressure and vacuum condition with defined, in-house organisms. It should not be linked to media fill pass or fail. Even with passed media fill CCIT may fail and require corrective action on container, closure and/or its integration process / machine parameters.

4 Purpose of this guidance

- As mentioned in the preface of this guidance, it provides current industry practice, answer to some frequently asked questions / thoughts and information on the process simulation (Media fill), scientific media fill design, methodology, and action plan in case of media fill failure with risk based approach.

5 Types of dosage form covered under this guidance

- This guidance applies to aseptic processing of finished dosage forms. Though not limited, this guidance is applicable for followings dosage forms:

    - Liquid vials
    - Ampoules
    - 3 pieces (plastic) bottle (Ophthalmic products)
    - Lyophilized process
    - Semi-Solid (Ointments)
    - Sterile Compounding Operations/Suspensions
    - New drug delivery system (NDDS) process e.g. liposomes, microsphere, depot products etc.
    - Dry powders
    - Infusion Bags
    - Drug and Device combinations e.g. Cartridges and PFS
    - Aseptic and Heat treatment combination

6 Risk assessment of aseptic process simulation (APS) and media fill plan

- Prior to media fill, a risk assessment is required to determine, identify, and evaluate the aseptic process steps and interventions (type, frequency and duration) that can potentially adversely affect the sterility assurance risk to the product.

- Risk assessments can also be used to determine the worst case manufacturing scenarios related to container size, configuration, line speed, batch size, and operating conditions. Where feasible, efforts should be made to mitigate identified risk by eliminating or changing risky process steps, and improving facility, equipment, and process design. The number and type of aseptic process simulations performed should be based on an assessment of risks posed by the process or significant changes to the process.

- Note: No high risk aseptic operation and/or high risk manual aseptic operations should be justified with a passing / successful media fill. It must be understood that we need to reduce the risk by designing process to the best extent feasible in a aseptic processing set up. Example: If we observe no. of interventions on a filling line due to vial toppling is 35 times in total filling duration of 6 hours. To justify these, if we simulate not less than 35 times vial toppling in a media fill of same process then it is not a good approach. Any intervention possess a risk to aseptic process. If such toppling challenge is more frequent, then instead justifying it with successful medial fill, it must be investigated to find the root cause and the same to be corrected.

- Manual aseptic processing involved greater risks than automated aseptic processes. A risk-based quality risk management approach can be helpful. It is therefore essential that there is a thorough and complete understanding of the process, including critical steps, and risks.

- **Refer Appendix I as a specimen to risk assessment of the aseptic line.**

7 Conditions when media fill is required and how many runs?

A. Start-up media fill (Initial validation of an aseptic process):

- Start-up / initial validation media fill is required for a new aseptic facility and/or new introduction of any aseptic production process and/or on introduction of new aseptic processing equipment e.g. filling line

- Process simulations are performed as part of the overall validation activities. At least three (3) consecutive successful process simulations (media fill) are performed when qualifying a new facility, filling line or process before starting the routine manufacturing.

- Bracketing of media fill can be performed for same filling line with similar aseptic process with worst case container closure and process determinations. Details are mentioned as below.

- Each new type of aseptic process, facility or line must be validated with media fills prior to use for aseptic drug products processing on same facility/line.

- Process simulation tests should be performed as part of the initial validation, with at least three consecutive satisfactory simulation tests that covers all working shift in which the aseptic process may occur in.

    - Three media fills with smallest container with widest mouth and highest speed of concerned container closure.

    - Three media fills with largest container with widest mouth, with slowest speed, including separate filling set-ups*, are required to ensure that the results are consistent and meaningful.

    - *Note: Each media fill run should consider a separate filling set up. In same filling set up continuation of another media fill run is not an acceptable practice. Similarly use of different speed in same medial fill is NOT a good approach. Speed to be determined as worst case and a single speed to be maintained throughout media fill.

- Additional run may be necessary depending on complexity of process, filled unit sizes and types, line configuration and other variable that may require additional runs to bracket production processes and / or complete worst case evaluation.

- Bracketing/matrix approach and its risk assessment shall be prepared to document the decision process and scientific rationale for the number of required media fills on a given production fill line. Matrix shall be prepared based on product container and closure configuration which are to be filled on the particular line as per production and/or exhibit batches.

- In case of intermediate size of containers and closure configuration falling within bracketing matrix one successful run shall be taken for each size of container (based on risk assessment, in certain case no media fill is required for the container and closure falling within the matrix for same aseptic process and line). Bracketing matrix shall be revised based on any new introduction of container/closure configuration. For introduction of new container or closure, which does not fall under bracketing matrix of the media fill at least three successful consecutive media fill shall be conducted.

- For introduction of any aseptically filled product (new product), assessment to be carried out for the evaluation of aseptic process flow encompasses in that particular product and on the basis of evaluation of risk assessment it requires to decide whether it trigger media fill for that particular product or not.

- Initial process simulation study should be started only when all pre-requisite activity e.g. utility, equipment, facility qualification and relevant trainings are completed to start media fill.

B. Periodic Media fill (Periodic requalification of aseptic process):

- Each aseptic product processing line required to have a “periodic” media fill. Frequency should be at least every six monthly. At least “one satisfactory media fill simulation run shall be executed on each operational line, when results are successful and consistent with initial validation". i.e. under the condition, when there were no changes in the normal production procedures/facility.

- For periodic media fill, an approach may be applied to perform process simulations for processes, container sizes on a rotational basis, with each process challenged periodically and/or on based on the risk assessment.

- Example: Smallest and largest containers shall be considered along with batch size to be adequate to mimic commercial production conditions to appropriately assess the potential risk involved in commercial batches.

- Smallest and biggest containers shall be simulated alternatively for scheduled re-qualification of the line. Other container closure configuration to be rotated along with the worst case container closure configuration to cover within 5 years or as appropriate.

- A specimen/example of rotational media fill schedule for different container size to cover smallest, largest container (worst cases) alternatively in every six months and other container size at least once in 5 years is given below (where 5ml and 100ml are smallest and largest containers respectively).

Container size	Initial	6 M	12 M	18 M	24 M	30 M	36 M	42 M	48 M	54 M	60 M	66 M
5 ml	√	√		√		√		√		√		√
10 ml			√									
20 ml					√							
30 ml							√					
50 ml											√	
80 ml								√				
100 ml	√		√		√		√		√		√	
M = Month						*√ = Media fill to be performed*						

Note: This is an example. Based on risk assessment the frequency of covering different container size within the bracketing matrix to be determined by the user.

8 Additional media fill:

- Additional Media fill may be required based on a risk assessment to assist in the evaluation of any major changes to procedures, practices or equipment configuration and before and after major modification and long shut down of aseptic processing line.

1. Modification/ Changes

- Before any modification / changes (even for improvement) which may impact aseptic process, then media fill to be conducted on any of the worst case container size for worst case aseptic process. Single run may suffice the requirement for such cases. Even if the change is predicted and/or for improvement of the aseptic process, a closing media fill is required. Because, any previous impact on aseptic process/line prior to changes may get masked after the change. Hence such closing media fill is required.

- After any significant modification to operational practices, facilities, services or equipment (e.g. modification to the HVAC system, equipment, major/long facility shut down, changes to process, change in number of shifts and change in numbers of personnel etc.), media fill is required. I.e. based on risk assessment.

- In such revalidation media fill, the number of runs to be decided with risk based approach. Under major changes, if it is decided to take three media fill runs, these three runs should include one run each of worst case container, closure size (one with largest and one with smallest container size with widest mouth for both) and one run of most frequently used container size on particular line in case of any of the below mentioned activities has been carried out. Like this any bracketing approach, if used, should be documented with risk assessment and scientific rationale.

- Each firm should determine the frequency of and interval between ongoing media fill (APS) for each process considering local and/or regulatory requirements, as well as additional risk based criteria, such as line design and performance.

- Media fill is also typically performed after an extensive maintenance event, such as a major facility shut down, which results in risk to the satisfactory performance of aseptic control systems. However, no media fill is required for minor maintenance work of area and/or AHU e.g. Epoxy, AHU maintenance etc. In such cases validated area recovery process to be followed. E.g. extensive cleaning, sanitisation, followed by physical environmental parameter verification. Viable monitoring can be ongoing with actual process for such minor maintenance / few days AHU maintenance works in aseptic area.

- In case of major civil work or changes, an APS will assist in verifying that the area has been returned to a qualified state and is acceptable for resumption of routine production. If these processes differ significantly, then supporting APS should be performed for each process.

- Previous simulation studies would no longer be representative or applicable, for following a process change(s):

- Examples of such changes include:
    - Changes in the process/ line configuration impacting sterility assurance.
    - Modifications to the equipment (interchanging identical standard parts does not constitute an equipment modification)
    - Modification to equipment or facilities that potentially affects the air quality or airflow in the aseptic environment
    - Major changes in the number of production personnel or initiation of second (or third) shift production when the facility has been qualified only for single shift operations.
    - Major changes to the aseptic production process and/or procedures
    - Major modification to the equipment preparation or assembly techniques
    - The addition of new product containers or container-closure combinations (Not falls under bracketing approach)
- Risk assessment to be performed to document the decision process and scientific rationale for the number of required media fills on a given production fill line.
- It also may be necessary to re-qualify a fill line with acceptable process simulations after corrective action(s) have been implemented in response to adverse trends or failures in the on-going monitoring of the facility or process, such as:
- In case of total filling time of the new product is greater than validated filling time for the batch size of the media fill taken on the filling line.
- In case of any persistent presence of strict anaerobic organisms has been confirmed in either environmental monitoring in aseptic area or, more likely, during end product sterility testing, media fill shall be performed with suitable media that supports growth of strict anaerobic organism.
- If processing lines stand idle for more than 6 months/ shut down.
- Note: Risk assessment may be beneficial to present a rationale for the need, number of runs and extent of aseptic process simulation (Media fill) as a result of change control. The risk assessment and risk management decisions should be recorded, approved and incorporated into the change control documentation.

2. Potential failure: Whenever there is evidence of a failure to maintain product sterility.

- Continued critical area environmental monitoring results above the alert/action levels

- Anomalies in environmental monitoring result. (Continuous out of trend found during the environmental monitoring)

- Failure in sterility test (Based on the investigation recommendation and root cause of the sterility failure)

- As per recommendation during any event or investigation.

- In case of growth promotion test failure and investigation.

- When an investigation fails to reach well-supported, substantive conclusions as to the cause of the media fill failure, then revalidation to be considered with at least one worst case container size and minimum three consecutive successful runs of same container and closure system, process in which media fill failed.

- If failure is in multiple container closure type of same process line in a campaign, then at-least one run of each container and closure along with one run of one of the worst case container size cumulatively not less than three successful runs shall be considered in line with increased scrutiny of the production process may be performed.

- Media fill failure demands a re-validation depending on the result and conclusion of the follow-up investigation. This re-validation may require the inclusion of one to three satisfactory process simulation tests (Media fill).

3. Closing Media fill:

- A closing media fill need to be performed on an APS (Media fill) after the last commercial batch prior to major/long term (more than 6 month) shut down without any changes to line/process i.e. the nearest next schedule/periodic media fill to be preponed and to be performed prior to such shutdown e.g. In case of more than 6 months of inactivity/hibernation planned or before decommissioning or relocation of a line/ major modification.

9 Pre-requisites for media fill

- Prior to initiating the Process simulation confirm satisfactory of the qualification, validation and operation procedure in-place of aseptic process support and sterilization systems, including (but not limited to):
    - Utilities qualifications (Water system, Compressed air, product contact gases)
    - Disinfectant qualification
    - Cleaning and sanitization program
    - Area qualifications including HVAC
    - Temperature, Humidity and Differential pressure control and its monitoring
    - Viable and non-viable control and EM monitoring program
    - Equipment and line qualifications
    - Personnel training and Qualification (OJT)
    - Personnel gown certification (qualifications)
    - Personnel gowning and de-gowning procedure
    - Material disinfection control
    - Material movement procedure
    - Product, container/ closure, product contact equipment sterilization/ De-pyrogenation
    - Air flow pattern/ Smoke study
    - Container closure integrity
    - Approved vendor for media
    - Protocol for media fill/ APS
    - Vendor qualification for the media

- GPT of media used for media fill and monitoring
- Availability of media decontamination agent
- Incubators qualification (For desired incubation condition)
- Protocol for Media fill
- Training on the protocol
- Visual inspector qualification

10 Design of media fill (APS)

- A media fill should be carefully designed to ensure that the simulation is representative of all the aseptic manipulations, its frequency and durations performed during production process.

- These include preparation and assembly of the product containers, transfer of the product containers to the fill area, and all process steps downstream from the “sterilizing filter” up to product filling, sealing/capping in finished product/simulated containers.

- Finished product containers with medium should then be incubated to permit the growth of microbial contamination in any containers.

- Microbiologically contaminated containers are expected to exhibit observable evidence of microbiological contamination after suitable incubation.

- The same type, size, design, method of cleaning, sterilization, depyrogenation to be followed along with same specification of containers to be same to the best extent feasible as used in routine production.

- Media fills should be conducted in the same locations where the production occurs and employ the broadest scope of possible manipulations that could occur during production and matching with airflow/smoke pattern study.

- Every aseptic manipulation during production up to the point of finished product release should be included in the media fill. Because each finished product container is to be sampled aseptically prior to release, sample withdrawal and any adjustments should be simulated as well and no sample to be discarded. All such samples which are actually discarded during filling operations must be incubated with identity of interventions as population 2 for information.

- The media fill is an experiment and therefore should include controls. These controls are independent of the quality audit of the growth medium (i.e., growth promotion testing).

    - A positive control for a media fill is a sealed product container of medium that is inoculated with a small number (i.e., less than 100 CFU) of microorganisms. Inoculation of the positive control container should be done in an area separate from the critical manufacturing area.

    - To ensure the absence of false positive results, a negative control should be included to demonstrate that the medium was sterile to begin with.

    - A negative control may be prepared by pre-incubating the medium, or by aseptically transferring medium into a separate suitable sterile container and incubating the control simultaneously with the media fill test containers.

    - These controls may not need to be repeated when multiple media fills are being done in a campaign and use the same lot of growth medium

- All steps intended for aseptic manufacturing should be reproduced in the media fill, including sampling and dilution of the final product.

- All personnel involved in the aseptic manufacture of the drug product should participate in at least one media fill per year.

- All processing steps that the operator normally performs during aseptic manufacturing should be simulated.

- The simulation process should duplicate the actual production process where the aseptic steps are conducted, from the set-up of the vial// assemblies to the transfer of the bulk drug from the sterilizing filter into the final containers that are ready for release.

- If the process is expected to include the addition of sterile diluent to adjust the strength following product assay, sterile medium should be added in the same manner during the media fill.

- The temperature of the medium should be conducive for growth of organism in the medium and should not chill / freeze even if actual production process has freezing / chilling.

- After the final product container is filled and ready after initial leak test, visual check, then it should be incubated in a temperature-controlled incubator.

    - Incubation between 20 –25° C for at least 7 days followed by examination containers by qualified personnel in a qualified incubator with data recording facility for temperature. Thermal validation of such incubator / walk in chambers to be done with and without load condition. Monitoring of temperature to be done at worst case location and probe to be placed inside the trays containing containers i.e. as per validated process/SOP if required.

    - Thereafter incubation between 30 –35° C for at least 7 days followed by examination containers by qualified personnel

    - Note: The incubation period of a media fill should be no less than 14 days. The containers shall be examined before incubation and before change to next incubation condition (7th Day) and end of the incubation (14th days)

11 Interventions:

- Activities performed by personnel/ operators in proximity to the aseptic fill zone, also called interventions. Humans e.g. operators/ personnel present in aseptic areas are the greatest source of microbial contamination during an aseptic process.

- The process simulation testing/ Media fill should consider the aseptic manipulations and interventions known to occur during normal production as well as worst case situations.

- The execution of interventions during the Media fill / APS is critical to the process capability demonstration. To demonstrate that capability, Media fill should include all the interventions that occur during an aseptic filling process along with its frequency and durations with worst case approach.

- Interventions that are permitted in a routine production operation should be specifically documented and included in media fill simulations at the same frequency and time duration. Practice for the handling of the Interventions should be performed as same performed in Smoke study/ Air flow pattern study. Interventions that would represent an unreasonable risk of contamination should not be included in either process simulation or routine production.

- The performance of interventions should be accomplished by qualified personnel, including maintenance personnel per the defined procedures. The ability of the operator/ mechanic to intervene in the process to fix a “mechanical failure” should be simulated in the APS. For example, a firm may choose to simulate an equipment breakdown. However, it is difficult to predict the frequency of occurrence of breakdowns, part replacements, or other non-routine corrective interventions. If these types of corrective interventions do not occur naturally during a process simulation study, the activities associated with them must be simulated to qualify their performance during routine operations.

A. Type of interventions

- There is two type of interventions:

    - Inherent Interventions (Routine Interventions): Inherent interventions are normal and planned activities that occur during an aseptic filling process (e.g., equipment set-up, weight adjustments, closure addition to hopper/bowl, container addition to hopper/bowl, EM sampling, etc.). Inherent interventions are not corrections to events that occur on the filling line. They are a planned and documented part of the overall process and are performed during the media fill at a defined frequency, duration or point of the filling/aseptic manufacturing operation.

    - Corrective Intervention (Non routine Intervention): Corrective interventions are performed to correct or adjust an aseptic process during its execution. That is not part of the planned aseptic process, Corrective interventions include; container breakage, tip-over of a container, stopper jam, change in filling needle, change in filling equipment, dose adjustments/samples, clearing automatically rejected units, etc. Since corrective interventions are un-planned, they should be clearly identified and documented in the associated records for its frequency and durations.

B. Identification of the interventions

- The identification of interventions, type and their frequency and time duration may be determined from a review of completed batch records, batch related documentation and discussions/ brain storming with operating personnel/ cross functional team and process mapping. The goal of this activity is to list all interventions for each circumstance. The basis and number of the required simulated interventions must be documented. The media fill should include a defined and representative number of inherent and corrective interventions along with its durations that can be expected to occur during an actual production operation. Inclusion of corrective interventions in successful process simulations can demonstrate acceptable aseptic technique and control.

C. Interventions evaluation through water trial/placebo trial

- It is recommended to perform the water trial/ placebo trail/ engineering trial to evaluate / understand the aseptic processes of the new facility, new processing line/ skid / new technology (New drug device combination, novel aseptic drugs, isolator etc.). It is essential to determine and identify expected interventions, types of interventions, their frequency, duration, operator manipulations, handling of the intervention, material movement and constrains if any. All the identified interventions are documented and incorporated in the media fill protocol to simulate the same interventions with the same frequency or expected frequency during routine production.

- There should be an authorized list of allowed interventions, both inherent and corrective, that may occur during production. The procedures listing the types of inherent and corrective interventions, and how to perform them, should be updated, as necessary to ensure consistency with the actual manufacturing activities. In the event that an unauthorized/ new intervention is required, details of the intervention conducted should be recorded and fully assessed.

D. Protocol for review/re-establishment of intervention based on every 6 monthly/25 batches data (whichever is more):

- The firm has to define the SOP/ protocol for ongoing intervention trending and evaluation for identification of the intervention, duration and frequency of the intervention during commercial batches.

- *Refer the Appendix II: Interventions trending and its evaluation.*

- Based on the trending of the interventions, identify the new interventions (if any), its maximum duration and frequency of the intervention, which might not simulated in the initial media fill runs, then media fill protocol to be revised to incorporate the same for simulation of the proposed interventions and its maximum duration and frequency. Whereas based in the trend data interventions and its maximum frequency and duration already simulated in the initial media fills/ previous media fills, then media protocol revision for the same is not required.

- It is recommended to prepared dynamic protocol to identify and document the new interventions and its frequency and time duration, which are not identified and simulated in the start-up media fills (initial media fill) for new facility/ new process lines. This protocol can be executed at-least every 6 monthly or 25 past batches whoever is higher to establish and determine the interventions, observed during the routine production and ongoing media fill. In the event that an unauthorized/ new intervention is required, details of the intervention conducted should be recorded and fully assessed for impact assessment.

- A new corrective intervention/ interventions performed during a routine aseptic filling must be evaluated. The intervention may be determined acceptable if it is similar to a previously simulated intervention and was performed with proper aseptic technique. Risk assessment should be performed for evaluation of such a new corrective intervention for impact assessments.

- Documentation of a new corrective intervention/ intervention should be reviewed during the batch disposition and review process. This review should determine the extent to which the intervention was a deviation from the routine manufacturing process and the acceptability of the intervention itself. The assessment should conclude with either an acceptance or rejection of this intervention relative to the current and future manufacturing processes. If the new practice is accepted then it should be reviewed for inclusion into the list of identified interventions simulated during a scheduled media fill (Ongoing).

- Note: Any atypical excessive intervention of a line / process due to a special reason/deviation may not require to be considered that as a new requirement to have those frequency, duration qualified for routine intervention. Rather the root cause of such intervention to be corrected. Such excessive intervention simulation in media fill can be performed for one time as concurrence to the risk evaluated during concerned product release if any.

12 Process Simulations (Media fill):

A. Process simulation plan

- Following points to be considered for developing process simulation plan:

  - Identification of worst case conditions covering the relevant variables, such as container size and line speed, and their impact on the process. The outcome of the assessment should justify the variables selected.

  - Determining the representative sizes of container/closure combinations to be used for validation. Bracketing or matrix approach may be considered for validation of the same container/closure configuration for different products where process equivalence is scientifically justified.

  - The volume filled per container should be sufficient to ensure that the media contacts its entire product contact surfaces when inverted or swirled. The volume used should provide sufficient headspace to support potential microbial growth and ensure that turbidity / color change can be detected during inspection.

  - All equipment and component contact surfaces should get in contact with media that may directly come in contact with the sterile product manufactured aseptically.

  - Maximum permitted holding times for sterile product and associated sterile components and equipment exposed during the aseptic process.

  - The method of detection of microbial contamination should be scientifically justified to ensure that any contamination is detectable.

  - The selected nutrient media should be capable of growing a designated group of reference microorganisms as described by the relevant pharmacopeia and suitably representative local isolates and supporting recovery of low numbers of these microorganisms.

  - The requirement for substitution of any inert gas used in the routine aseptic manufacturing process by air unless anaerobic simulation is intended. In some situations, where anaerobic organism found/process conducive to anaerobic organism, then inclusion of occasional anaerobic simulations as part of the overall validation strategy should be considered.

  - The process simulation should be of sufficient duration to challenge the process, the operators that perform interventions, shift changes and the capability of the processing environment to provide appropriate conditions for the manufacture of a sterile product.

  - Where the facility operates in different shifts then the APS should be designed to capture specific factors (e.g. for those manufacturing during a night or extended shift, fatigue should be considered).

  - Simulating normal aseptic manufacturing interruptions where the process is idle (e.g. shift changeovers, recharging dispensing vessels, introduction of additional equipment, etc.).

- Ensuring that environmental monitoring is conducted as required for routine production, and throughout the entire duration of the process simulation.

- Where campaign manufacturing occurs, such as in the use of robust Barrier Technologies (isolator etc), Blow Fill Seal and/or manufacture of sterile active substances, consideration should be given to designing and performing the process simulation so that it simulates the risks associated with both the beginning and the end of the campaign and demonstrating that the campaign duration does not pose any risk. The performance of "end of production or campaign APS" may be used as additional assurance or investigative purposes; however, their use should be justified in the contamination control strategy and should not replace routine APS. If campaign process used, it should be demonstrated that any residual product does not negatively impact the recovery of any potential microbial contamination.

13 Start Point and End Point of Process Simulations/ Media fill (excluding incubation and interpretation of result)

- APS starts from the point of product and component sterilization to closure of the container (including any process/handling steps subsequent to sealing that might impact container integrity).

    - Start point: Point of product and component sterilization, preparation/transfer of pre-sterile items(if any) onward

    - End point: Closure, treatment of the container (Sealing, Leak Test etc. i.e. if that might impact on container integrity). Note: If external washing process is there, then the same to be followed with the cleaning medium (e.g. water for injection/purified water) temperature between 20-25 °C.

    - For sterile bulk materials, conduct process simulations for from the point of material sterilization through to the completion of bulk packaging.
    - Note: Process simulation process steps should cover for aseptic compounding steps, filtration, sterile material transfers, filling, Lyophilization (with loading, partial vacuum, unloading), closing and sealing, maximum exposure time for open containers on the line, and inert gassing (simulate using compressed air) etc. Media fill simulation is not required for the steps of non-sterile manufacturing and/or non-sterile processing parts except of cleaning, sanitization of area, facility, equipment and preparation of contact parts and components. Extra cleaning and/or additional sanitization than scheduled cleaning/sanitization must be avoided prior to media fill. It should be as routine.

    - No additional precautions, no additional checks, no additional check list than routine to be used for media fill. Media fill is to be equal or worst case simulation to routine aseptic operation. Because, media fill provides vital sterility assurance information to our aseptic process. If we take additional actions specifically for media fill, then the purpose of media fill will be defeated.

14 Process Simulations critical attributes

- Following are the critical attributes of the media fills/ APS:
    - Operator intervention/ Manipulation
    - Fill volumes/ Container sizes
    - Container closure system
    - Line speed/ Run duration
    - Aseptic processes
    - Number of containers
    - Operating shifts
    - Number of personnel
    - Hold times
    - Facility and design (Convectional LAF, RABS, Isolator etc.)
    - Handling of the interventions
    - Type, Frequency and duration of the interventions
    - Number of the container to be removed during interventions
    - Line set up and equipment connection
    - Media used for process simulation
    - Environmental conditions
    - Incubation and inspection
    - Acceptance criteria
- Careful consideration should be given to each of the above parameters for inclusion in the (aseptic process simulation) media fill study design. Parameters selection and its rationale and justification should be well documented and approved.

15 People's training and personnel qualifications

- Each person in the aseptic area (e.g., operations, engineering, quality any other) has the potential to introduce microbiological contamination. The risk to finished product sterility increases as operator activities increase in an aseptic processing operation. To ensure maintenance of product sterility, it is critical for operators involved in aseptic activities to use aseptic technique at all times. So appropriate training should be conducted before an individual is permitted to enter the aseptic manufacturing area.

- Fundamental training for operator should include aseptic technique, cleanroom behavior, basic microbiology, hygiene, gowning, cGMP training and patient safety hazards posed by a non-sterile drug product/ materials/ equipment.

- In addition to that operator should be trained on specific clean room operation, job specific training in his function area, relevant intervention handling training and the specific written procedures covering aseptic manufacturing area operations.

- Personnel must successfully meet the firm's gowning certification requirements. Personnel should be trained on the process simulation protocol.

- Personnel/ operator involved in the inspection of the media fill, they should have appropriate education, training, experience and must be qualified in inspecting media fill units for microbiological contamination. At the time of media fill inspection, each media-filled unit should be examined for contamination by these trained and qualified personnel/ operator.

16 Allocations of activities (Direct, Indirect and activity based)

- Operator has to complete his assigned training, relevant to his function area and job specific.

- Operator and personnel, who directly involved in the critical operation and perform interventions under the critical zone, consider as direct involvement and they should have to follow the strict aseptic practice and appropriate aseptic behaviors in the area during manipulation and intervention handling.

- Whereas the personnel, who supports the operators performing the critical operation and intervention, and not directly involved and access critical zone are considered as indirect involvement.

- Apart from these, some of the operators, who are responsible for area cleaning and sanitization, supervision and quality oversight and not involved directly or indirectly in the aseptic manipulation and interventions in critical zone.

- There will be supervisor who may only supervise the activity and not involved directly.

- Hence, for media fill the operators and supervisors of various functions need be qualified by allocating same/similar action as they need to perform during routine production. These qualification to be documented and these personnel to be qualified activity wise.

- Examples: The operator who performs filling machine set-up/ assembly need to be qualified in media fill by actual performance of set-up/assembly during successful media fill. Similarly each operators who need to be qualified for various interventions, shall perform actual intervention in successful media fill of each type of critical interventions. The personnel performing indirect actions, supervision need to follow the same in media fill as well.

- Note: Just by entering inside aseptic area during a successful media fill never qualifies an operator/supervisor for aseptic interventions. For such qualification above to be followed.

17 Media selections

- Following criteria should be considered for the selection of the media for Aseptic process simulation/ media fill study.

    - Low Selectivity: The medium selected should be capable of supporting a wide range of microorganisms, which might reasonably be encountered and be based also on the in house flora (e.g. isolates from environmental monitoring etc.).

    - Growth promotion Test: Media used in the evaluation must pass a growth promotion test. The control organisms (Gram positive and gram negative bacterial, yeast and mold etc.) used should include those relevant strains of test micro-organisms identified by relevant Pharmacopoeias as being suitable for use in the growth promotion test.

    - It must show excellent growth performances and has to be safe and easy to use.

    - Growth promotion tests should demonstrate that the medium supports recovery and growth of low numbers of microorganisms, i.e. 10-100 CFU/unit or less.

    - Growth promotion testing of the media used in simulation studies should be carried out on completion of the incubation period to demonstrate the ability of the media to sustain growth if contamination is present. Growth should be demonstrated within 5 days at the same incubation temperature as used during the simulation test performance.

    - Clarity: The medium should be clear to allow for ease in observing turbidity. A suitable color media can be used as well.

- Medium Concentration: Recommendations of the supplier should be followed unless alternative concentrations are validated to deliver equal results.

- Filterability: If a filter is used in the aseptic manufacturing process, the medium should be capable of being filtered through the same end connection type/assembly similar and/or as used in production. MOC of filter need not to be same as product. However, its filter rating and design type to be same to simulate actual aseptic interventions for filter connections/aseptic handling.

- Media used for process simulations/ media fill study may be liquid or powder, depending on the type of filling process to be simulated.

- Media containing animal derived components should come from non-BSE/TSE origin. The most common medium for process simulation is Soybean-Casein Digest Medium (SCDM) or Trypticase Soy Broth (TSB).

- SCDM is a general purpose growth medium well suited for the recovery of aerobic microorganisms of the types commonly associated with human borne contamination. It is very similar to SCDA which is widely utilized for microbial recovery in aseptic areas for the same reason.

- However, there is media with color indicator commercially available, which facilitate the time of media fill container inspection to check the color change and growth. Also color media are requirement for translucent containers / plastic containers, small containers, containers with narrow lumen etc.

- Replacement of the products, diluents, and buffer solutions with media is customary when performing process simulation studies.

- Aseptic processing conducted in a strict anaerobic environment (one which maintains less than 0.1% oxygen throughout the process) should be evaluated with alternate Fluid Thioglycollate Medium (FTM) or other suitable medium, in addition to aerobic evaluation.

- An anaerobic media fill may also be considered for a typically aerobic process if anaerobic microorganisms are consistently recovered during periodic environmental monitoring (for anaerobes), or if facultative anaerobes are detected exclusively in FTM sterility test medium. In either case, oxygen is excluded from processing and parameters such as container fill volume and inert gassing may require modification to provide a true anaerobic environment for the aseptic process simulation study.

- The selection of placebo material for use in process simulation must consider several factors. The seemingly obvious choice of dry sterile media, itself, has proven less than successful because of its poor flow properties, which make its passage through conventional powder handling equipment or a typical sterile powder filling machine a considerable challenge.

- The most commonly used placebo materials which have been used successfully include: lactose, mannitol, polyethylene glycol 6000 and sodium chloride.

- The chosen material must be easily sterilizable (using a validated method/pre-sterile), dispersible or dissolvable in the chosen medium with minimal agitation, have no adverse effect on growth promotion, and be easily handled in the mock formulation processes or easily filled in the powder filling equipment.

- Note: Mycoplasma free media (as per Manufacturer's COA) shall be used for media fill studies.

Various nutrient media for process simulations are recommended by various guidance documents as below:

Guideline	Requirements
FDA Guidance on Sterile Drug Products Produced by Aseptic Processing	Before a medium is chosen for validation runs, it should be demonstrated capable of supporting microbiological growth. Generally, a microbiological growth medium that supports the growth of a broad spectrum of aerobic, microorganisms, such as soybean casein digest medium, is acceptable. The most important aspect of media is its ability to promote microbiological growth. Before any medium is chosen for process simulation runs, it should be demonstrated capable of supporting microbiological growth.
USP <1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environment	In general, an all-purpose, rich medium such as soybean casein broth that has been checked for growth promotion with a battery of indicator organisms.
Recommendation on the Validation of Aseptic Processes, PIC/S PE 002-1	The medium should have a low selectivity i.e. capable of supporting growth of a wide range of microorganisms.
Aseptic processing of Health Care Products - Part 1: General Requirements, ISO 13408-1	The media selected for media fill runs shall be capable of growing a wide spectrum of microorganisms and supporting microbiological recovery and growth of low numbers of microorganisms, i.e. 100 colony-forming units (CFU) / unit or less

- Medium from a qualified commercial vendor may be used by facilities for media fills. Shipping, storage, preparation, and handling procedures should be carefully designed, documented, and followed to ensure media integrity and stability.

- Commercially prepared media should be used within the label's shelf life and stored according to the label's recommendations.

18 Vendor Qualification (Media)

- The firm has to define the procedure for qualification of the vendor, engaged to manufacture and supply media. Which will be used for process simulation study (Media fill).

- The vendor qualification may include site visit/ audit and quality test of three different lots/batches. Three commercially available batches/lots of medium from a single supplier should be subject to quality control tests. These tests should include visible inspection, pH, sterility, and growth promotion. Results of this testing should conform to the results reported in the certificate of analysis (CoA). Many vendors will have samples of different batches available for purchase and testing. If three different batches are not available initially, then it may be necessary to test each of the first three incoming batches for conformance to the CoA.

- SCDM/ Media should be sterile (confirmed by incubating samples for 7 days at a defined controlled temperature), pH 7.3 (±0.2), and able to permit growth of selected aerobic species (e.g., Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa).

- Vendor qualification programs should periodically verify the full CoA and growth promotion capability to ensure continuing vendor reliability.

19 Batch Size considerations

- The following are general approaches to define aseptic process simulation batch size (number of units). Uniquely small or large batch sizes may require modification from the approaches listed below. Each company must determine appropriate rationale and approaches applicable to their unique operations. Below table can be referred as a guidance i.e. extract from various guidances.

Extract from various technical guidance / industry practices			
Production batch description	**Production batch size (# filled containers)**	**Minimum APS batch size (# of filled containers of media)**	**Recommendations**
Small scale	< 5,000 units	< 5,000 units	❖ APS batch size should be at least equal to production batch size.

Mid-scale	5,000 to 10,000 units	5,000 to 10,000 units	❖ APS batch size should be of comparable size to the production batch size. ❖ For high speed filling or with maximum size production batches, it may be appropriate to fill additional units in order to accommodate normal aseptic manipulations, interventions, and realistic simulation of the process.
Large scale	> 10,000 units	> 10,000 units A variety of approaches can be employed to evaluate the process.	❖ Please see the note below*
Manual Fill	Any amount	Same as production batch size	❖ APS batch size should be at least equal to production batch size. ❖ Entire manual filling operation represents an intervention which should be captured.

- Note: The most accurate simulation model would be the full batch size and duration because it most closely simulates the actual production operations.

- *For large production batches the following approaches may be considered for the APS batch size and approach. If production batch size is greater than 10000 units, then media fill batch size must be targeted for filling and incubating not less than 10000 units. It must be ensure that the batch size decided for the media fill should be adequate to allow simulation of all interventions (routine and non-routine) at the same frequency as in routine production batches and also for its duration. Filling duration should be not less than routine filling duration of the production batch. Actual filling shall be performed to cover initial, middle and end of the filling duration for all shifts. During and post to any interventions there must be actual media filling and same should be recorded. During the non-filling durations machine shall be under operation to simulate the length of the actual fill duration at determined fill speed for particular run i.e. Under this approach, the aseptic line (Conveyer, turn table, etc.), personnel, procedures, material and trolley movement (If any) and processing environment are fully evaluated but the number of media-filled units produced is limited.

- Note: Always, fill enough units to simulate interventions and for a period of time not less than the amount of time operators are required to spend working in the clean room without leaving for break. The organization has to perform scientifically sound risk assessment and document the rationale for reduced batch size for media fill design.

20 Duration of Media Fill

- The duration for an aseptic process simulation should be sufficient to adequately challenge the aseptic process, the operators that perform interventions, and the capability of the processing environment to provide appropriate conditions for the manufacture of a sterile product.

    - The duration of aseptic processing operations shall be a major consideration in media fill design. The most accurate simulation model would be the full batch size and duration because it most closely simulates the actual production operations.

    - Filling duration should be not less than routine filling duration of the production batch. Actual filling shall be performed to cover initial, middle and end of the filling duration for all shifts.

    - The APS should also be of sufficient duration to include a representative number of interventions which might occur during an actual production filling operation. Where they are part of normal operations, gown changes, breaks and shift changes should be simulated. Justification of the selected number of units filled, duration and yield should be included in the process simulation study design/ protocol.

    - The duration of the APS should be long enough to capture the potential microbiological impact of performing those interventions.

    - Simulation of all inherent intervention shall be done including consideration of their frequency of occurrence and duration, so that the interventions are equal to or more than expected interventions during actual production operations.

    - Line wise type, frequency, duration of interventions (Corrective and inherent) should be defined in media fill protocols. Type, frequency and duration of interventions being simulated in media fills shall not be less than the allowed during commercial production.

- Note: If during routine production, the frequency, duration, type of intervention is more/new to that of successfully simulated interventions in media fill, then investigation to be carried out for the root cause, impact evaluation shall be done and based on the impact evaluation decision on batch disposition to be taken. Though not limited, if the intervention is similar to qualified interventions and supported with data of environmental parameters, viable, non-viable particle count, depending on type of intervention, batch disposition decision to be taken. The new frequency, type, duration should be simulated in next media fill study for one time. However, corrective action to be taken to reduce that new interventions. If it is unavoidable, then to be included in routine / non-routine / as worst case intervention in media fill protocol.

21 Operating shifts

- Where the manufacturer operates different shifts then the APS should be designed to capture specific factors (e.g. for those manufacturing during a night or extended shift, fatigue should be considered). If the operation perform in all three shifts, then media fill study should considered and capture all three shift and shift changeover in the simulation. If initial qualification covered only one shift operation and routine production operation required additional shift for routine operation, then subsequent media fill should be consider for the simulation of the shifts, which expected for routine operation. Impact assessment should be performed for the additional requirement of the shift for routine operations.

22 Dispensing and Compounding of Media

- The aseptic compounding process simulation may be performed as a stand-alone activity or be fully integrated with the aseptic filling process as per actual production process. When integrated with the filling process no adjustment for the compounding is to be made in the acceptance criteria. Process simulation studies should assess all aseptic operations performed subsequent to the sterilization of the materials utilized in the process.
    - Aseptic steps for products that are solutions may be limited to set-up, sampling, and in-situ filter integrity testing (if any).
    - Suspensions, ointments and other non-filterable formulations may require a substantial number of aseptic steps (e.g., sterile powder addition simulation).
    - Processes requiring the addition of sterile powders should employ an acceptable placebo material in containers identical to those utilized in the process being evaluated.
    - Blending, milling and subdivision processes performed at a sterile powder facility require similar simulation.

23 Utilities

- Compressed air shall be used for media fill instead of nitrogen gas/ Carbon dioxide/ any purging of gases whenever it is used in the product.
- Nitrogen flushing in the filling tank before and after filtration shall be simulated using compressed air.
- The pre-filling and post filling nitrogen overlaying (if any) shall be simulated using compressed air.

- For aerobic media fill, air should replace the inert gas and be delivered by the same delivery system thus assuring the purge/transfer set-up and delivery considerations are fully considered in the simulation. For anaerobic media fill inert gas to replace oxygen / air.

- The sterility of the inert gas system is confirmed through filter validation, integrity testing, and sterilization of connecting lines downstream of the filter, not by means of the process simulation. The use of an inert gas with Soybean-Casein Digest Medium may inhibit growth.

- If it is necessary to use an inert gas for simulation of an oxygen free process, testing should confirm the ability of the inert gas/medium combination to support microbial growth.

24 Filter considerations

- The filtration system used to produce sterile product is validated independently of the process simulation and does not require further validation by virtue of a process simulation.

- If possible, the same filtration system and vessels employed for product manufacturing can be used; however, in many cases this is not technically feasible or practical. Aseptic filtration of the media shall be performed using 0.22 micron filter/ sterile grade as mentioned in media fill protocol.

- Note: For the filling line having both the product configurations (Without end point filtration and with end point filtration), media fill study shall be performed with both end point filtration and without end point filtration. Such media fills can be scheduled alternatively over one year (period to be defined based on risk assessment) in routine media fill program. The filtered media/ make can be different than routine production, however the end connections and aseptic practices/ sterilization method for filters must be same in media fill as that in routine production.

- The sterilizing-grade filter may not be identical to the one used for the product(s) being simulated, but it should be sized properly for the preparation of the required volume of media. The use of pre-filters may be required as media may contain a substantial amount of fine particles that may clog the primary sterilizing-grade filter i.e. even when in actual operations no pre-filter is used.

- It is critical to design media preparation systems so that the fluid path to the filling machine and aseptic connections required effectively simulate normal and typical production operations. Usually has a 0.22 µm pore size rating. It is preferable to sterile filter the media into a sterile holding tank prior to start of the fill, rather than hold non-sterile media for several hours. Media should be allowed to cool to <35° C before use in a process simulation.

- Note: If there is a bulk sterilization at any stages and media may lose its growth property with overheating during bulk sterilization, in such cases, alternative approach of filtering media(for sterilization instead bulk sterilization by heat) into bulk sterilization tank can be followed. Scientific rationale to be prepared for such approach.

25 Hold Time considerations

- The hold times of sterile equipment, sterile closures and filtered media shall be covered under worst case matrix and to be validated initially/ rotational basis as part of media fills.

- Preferably single hold time shall be performed initially in each run instead clubbing of hold time in same run. Any worst case may replace hold time in particular run to have meaningful assessment of worst case.

- Hold time program should be covered under worst case matrix with other worst cases. All hold time to be covered at-least once in 4 or 5 years or as appropriate. Critical hold time e.g. Filtered media solution, product contact parts, sterile components must be covered in initial media fill with staggering/rotating in different runs.

- The hold time shall be followed for different items separately in subsequent media fills. Hold time study shall include, but not limited to following.

    - Sterile filtration and filling equipment shall be hold for not less than its maximum hold time (e.g. 48 hours from completion of sterilization to start of use) for which equipment can be used after sterilization.

    - Filtered media solution shall be held for not less than specified hold time as specified in the batch manufacturing record prior to start of filling and/or SOP of the process being simulated.

    - In case of simulation of freeze dried products the total hold time of the media solution should not be less than the maximum allowable time between the freeze drying cycle start time and the time of completion of filtration.

    - Sterile dress and goggles shall be held for not less than its maximum hold (time period from end of sterilization to start of usage). The number of hours to be decided based on intended or existing hold period.

    - Simulation of lyophilized product to be done considering its worst case process flow (i.e. from vial collection unit to maximum path for the lyophilizer loading and unloading area) and length of both loading and unloading operations.

    - Hold time to simulate a Lyophilization cycle and rationale for the selection of the hold time to be prepared and approved.

    - Using room/equipment at the maximum time period after completion of sanitization/ sterilization (clean hold time). The hold time between compounding of the media and filtration of media into a sterile vessel should be minimized.

- It is also important that process simulations incorporate storage of sterile bulk drug substances or product and transport to other manufacturing areas. For instance, there should be assurance of bulk vessel integrity for specified holding times. i.e. as routine production may not be continuous over the time period hence need to be simulated for hold time and to be validated.

26 Container and Closure configurations

- When a particular container/closure configuration provides unique operating challenges (e.g., tipping, jams) and causes increased interventions, it is recommended that a separate process simulation be performed with that particular configuration. Clear containers of identical configuration may be substituted for opaque or amber containers to aid in the detection of contamination. Closures which require additional or significantly different handling/insertion methods should be considered in the study.

- Determining the representative sizes of container/closure combinations to be used for process simulation. Bracketing or matrix approach may be considered for process simulation of the same container/ closure configuration for different products where process equivalence is scientifically justified. Matrix shall be prepared based on product container and closure configuration which are to be filled on the particular line as per production and/or exhibit batches.

- It is recommended, that in case of intermediate size of containers and closure configuration falling within matrix, one successful run shall be taken for each size of container. Whereas new container and closure configuration, which does not fall under bracketing matrix of the media fill at least three successful media fill shall be conducted.

- Matrix shall be revised based on any new introduction of container/closure configuration.

- However, the firm has to conduct the risk assessment and document the decision process and scientific rationale for the number of required media fills on a given production fill line.

27 Container Size

- In general, process simulation trials should entail at least the filling of the largest and smallest containers on a given filling line based on a facility established matrix. Exceptions to this general rule occur when the same filling machine is used for different product presentations. In these instances, the flexibility of the filler may make it necessary to evaluate more than one set of large and small containers, because the filling set-ups are so different. For example, if filling another size container results in a process which is significantly changed (e.g., additional different high risk manipulation or different fill parts – peristaltic instead filling by rotary piston for other product), then that size container should be included in the study.

- Typically the smallest and the biggest size of the container filled on a particular line shall be challenged to demonstrate bracketing of the container sizes. However, intermediate size may be used when required. Use of such size needs to be justified.
    - Large Containers with widest mouth with slow speed (Maximum exposure)
    - Small containers with widest mouth with high speed (Potential to increase manipulation/ interventions).
- Bracketing should also consider the handling characteristics and different line speed for all containers processed on the line. E.g. A line with 2 small containers may include a tall 2 ml that is less stable then the small 1 ml container, therefore, the tall 2 ml would be worst case for handling and should be challenged during media fill.
- In case of different mouth diameter in the same size of containers the largest mouth diameter containers shall be challenged because of the larger area exposed to the aseptic environment.
- The smallest container size can be challenging due to greater number of intervention as a result of container/ closure handling problems.
- In case of colored containers or opaque containers, suitable transparent containers of the same size shall be selected for media fill to the extent feasible. E.g. If product is in amber vial/ ampoule, clear vial/ ampoule of same size shall be selected for the media fill. In case of plastic bottles semi-transparent bottles shall be used. However, same product containers can be used where the contamination can be detected by visual inspectors with use of color media.
- In case of change of sterilization method of container and closure at least one successful media fill shall be executed. If the container closure are within matrix for any worst case of worst case of container closure three successful runs shall be executed.
- Risk assessment or Matrix approach perform to document the decision process and scientific rationale for the number of required media fills on a given production fill line. Matrix shall be prepared based on product container and closure configuration which are to be filled on the particular line as per production and/or exhibit batches.

28 Filling Speed

- Media fill should use only a single speed. The filling speed selected must be justified.

- Speed of the machine shall be specified in the Media fill protocol in term of vials/ minute, ampoules/ minute, bottle/ minute, Prefilled syringes/ minute, cartridges/minute for each speed.

- The filling machine speed for all the products need to be listed in the media fill matrix and need to be simulated during media fill as defined in the media fill protocol.

- It is recommended to use single speed either highest or lowest alternately for the simulation in the different media fill runs/ batch as used for filling of the commercial routine batches as below rationales.

    - Lowest Line Speed (Large Containers with widest mouth): The lower speed of the line is challenging due to prolong exposure of sterile drug product and container closure in aseptic environment. The lower speed may be simulated during media fill to simulate the longer exposure. Containers filled during lower line speed shall be identified appropriately

    - High speed (Smaller containers with widest mouth): High speed can be most challenging due to frequent number of intervention or a significant degree of manual manipulation as a result of container/ closure handling problems. The higher speed may be simulated during media fill to simulate the greater number of interventions arising because of the speed. Containers filled during higher line speed shall be identified appropriately.

29 Fill Volume

- The container need not be filled to its nominal fill volume. The fill volume must be controlled and monitored as performed during routine filling.

- Where partial fills are employed, Regardless of the actual fill volume, the process simulation should include a fill weight/ volume adjustment using methods identical to those employed during production. While the specific amount of medium utilized in a partial fill may not be critical, there are two general criteria:

- First, there must be enough medium in the container to contact all the container-closure seal surfaces when the container is inverted and swirled.

- Second, there must be enough medium in the container to allow for the detection of microbial growth.
    - The fill volume of media should be sufficient to wet the entire surface including the closures and to allow easy inspection. A volume of at least greater than 50% of the overfill capacity of container is recommended including lyophilized products. If the fill volume is less than 50%, a proper justification should be provided in the protocol.
    - The technique used to wet all surfaces must be defined in a protocol, e.g. inverting containers to wet surfaces. The containers shall be inverted before and during incubation. E.g. at least before starting incubation and after 7 days of incubation.
    - Note: Fill volume during media fill study should be selected considering qualified fill volume on filling line with respect to container size. It should be minimum 50% of container size and sufficient to wet entire surface when inverted and swirled during inspection.

30 Worst cases

- Identification of worst case conditions covering the relevant variables, such as container size and line speed, and their impact on the process. The outcome of the assessment should justify the variables selected.
- The use of “worst case” situations is intended to challenge the process under conditions that may be on the edge of normal operating conditions. If, under the circumstances of the worst case challenge, acceptable results are achieved, then there is greater confidence in the reliability of the system under more routine conditions. Worst case does not mean creation of artificial conditions or environments which exceed allowed operating conditions and which can force a system failure. Worst case conditions vary depending on the operations or risk being considered.
- For example, executing the APS using the maximum number of personnel may be worst case at certain times as gowned personnel are the greatest source of microbial contamination in an aseptic process. In other situations worst case may include executing the process with fewer people if this results in more movement by the process operators.
- Other examples of “worst case” practices may include:
    - Using room/equipment at the maximum time period after completion of sanitization/ sterilization(clean hold time)
    - Using the slowest fill speed for the largest container (maximum opening)
    - Using the highest fill speed for the smallest container (handling difficulty)

- At least the same number of personnel perform the media simulation run as would normally be present in filling a product. The number of personnel is to be specified in the media fill procedures for the media simulation, and documented on the media records for each run.

- The maximum number of personnel allowed to be present during aseptic filling operations (Product) must be covered under worst case during media fills as per worst case matrix on rotational basis and challenged during aseptic process simulation.

- The worst case conditions selected for inclusion in an APS should be predefined based upon characteristics of the operation. The identification of appropriate worst case conditions should be accomplished by conducting an assessment of the APS covering the relevant variables and their microbiological impact on the process. Such assessments can benefit from the application of risk management principles. The assessment conclusion should outline the variables selected as worst case and considerations/rationale for their selection.

31 Routine Interventions simulations

- 100% of all routine/inherent interventions must be carried out in every media fills. Number of times, duration of simulation for each intervention must be defined in media fill protocol or BMR. It should not be less than allowed number of interventions, duration for particular type during production batches.

- As mentioned earlier in this guidance, inherent interventions are normal and planned activities that occur during an aseptic filling process (e.g., equipment set-up, weight adjustments, closure re-supply, container re-supply, EM sampling, etc.). Inherent interventions are not corrections to events that occur on the filling line. Rather they are a planned and documented part of the overall process and are performed during the APS at a defined frequency or point of the filling operation.

- Though not limited, some examples of routine-interventions are as below:

    - Volume checking (Wherever applicable) and sealing height adjustment. The destructive fill volume checks in production batch shall be simulated without removal of the containers out of the aseptic filling zone. I.e. simulation to include container removal action without actual removal of the containers. All such containers shall be incubated as population 1. Actual volume is to be confirmed post 14 days of incubation for yield reconciliation purpose by collecting containers from initial, middle and end stage of filling for quantity not less than 5 times the total number of filling heads. Gross weight to be checked for the containers collected.

    - Simulation of in process checks like collection of initially filled container for pH checks will be done without actual removal of containers Any leftover bulk in filling tank to be collected in sterile container directly through the filling needles and shall be incubated as population 1. However any other sample directly collected from the tank/buffer vessel/filter, etc. manually into sterile container closure to be incubated as population 2.

- Rubber stoppers, plastic bottles, plug inserts and cap addition at same frequency as per routine production. It can be simulated to make same or more number of interventions than routine production batches.

- Sterile powder/media addition in case of skid manufacturing process as defined as per respective media fill protocol.

- Entry of environmental monitoring operator in the filling room during filling operation and performing the environmental monitoring during the media fill.

- Simulation of exposure of the half stoppered filled vial to pressurization and partial evacuation of the freeze dryer(FD) chamber in a manner that is representative of process steps in case of media fill with Lyophilization.

- Tub loading-unloading in pre-filled syringes filling machines.

- Simulation of sample collection for moisture check in case of FD products. Do not remove any sample. Only door opening, sample collection simulation without actual sampling shall be performed.

- Simulation of opening lyophilizer door throughout loading and filling operation. Transportation of half stoppered filled vials through the Mobile LAF trolley or through suitable device wherever it is applicable e.g. automatic loading- unloading system, simulation of FD unloading and transfer to capping also to be performed as well with consideration of speed and duration.

- Simulation for hopper loading for sterile ready to use cartridge in cartridge filling machine.

- Simulation of operation of swing conveyor (where applicable) same or more numbers of interventions than routine production batches.

- Simulation of rubber stopper, plug insert, cap, bottle and combi seal jam in chute and vibrator bowl, vibrator bowl/ chute adjustment, empty/ filled container fallen on conveyor belt or conveying device.

- Simulation of alignment of stoppering station (star wheel, pick and place unit) for stopper on half stoppered vial.

- Stoppage of the machine and restarting after shift change/ replacement of the operator with a new operator at the time of shift change if the media fill is in more than one shift.

- Simulation of o-RABS interventions and open door interventions.

32 Non-routine Interventions simulation

- 100% of all non-routine interventions are to be simulated in each media fill for its type, frequency and duration. Non-routine/Corrective interventions are performed to correct or adjust an aseptic process during its execution. While not part of the planned aseptic process, they are well understood operations and are recognized to sometimes occur during processing.

- Corrective interventions include: container breakage, topple of container, stopper jam, break down etc.

- Since corrective interventions are un- planned, they should be clearly identified and documented in the associated records. The APS should include a defined and representative number of corrective interventions that can be expected to occur during an actual production filling operation. Inclusion of corrective interventions in successful process simulations can demonstrate acceptable aseptic technique and control. A new corrective intervention (e.g., one not included in the firm's process simulation program) performed during a routine aseptic fill must be evaluated prior to batch disposition.

- The intervention may be determined acceptable if it is similar to a previously simulated intervention and was performed with proper aseptic technique. The evaluation of such an intervention may include an aseptic process simulation subsequent to the fill in which that intervention occurred. Evaluation of such a corrective intervention should be supported by a risk assessment. Documentation of corrective interventions in the batch record will allow for identification and trending of interventions occurring during production. Documentation of a new corrective intervention should be reviewed during the batch disposition and review process. This review should determine the extent to which the intervention was a deviation from the routine manufacturing process and the acceptability of the intervention itself. The assessment should conclude with either an acceptance or rejection of this intervention relative to the current and future manufacturing processes. If the new practice is accepted then it should be reviewed for inclusion into the list of identified interventions simulated during a scheduled APS. However any new intervention due to special cause/deviation if any to be simulated once in nearest scheduled media fill and corrective action to be taken to prevent such interventions.

- Examples of non-routine interventions are:

    - Intervention for overload of star wheel, worm of container carriage system, container carrying rack, simulation of alignment of bevel gear on the top of the syringe head (if any).

    - Entry of the maintenance person into the filling room, when the filling operation is going on and performing the maintenance activity in a simulated during the filling.

- Maintenance activity simulation: It will be simulated to assess the ability of the operator/ mechanic to intervene the process to fix a "mechanical failure" for the type of intervention at the respective stage of aseptic processing which will differs from line to line based on the line configuration.

- The maintenance activity simulation to be mentioned in the media fill protocol and impacted containers (which are supposed to be discarded as per SOP/procedure if any) are to be incubated (as Population 2 with proper identification linked to this intervention). This intervention should be clearly specified and documented in BMR/media fill protocol.

- Quantity of the impacted container due to maintenance activity and line clearance shall be mentioned in respective media fill protocol of filling line.

- Duration for the maintenance simulation to be defined and the same is to be simulated e.g. 3-4 hours or as appropriate as expected during operation.

- For e.g. Syringe clutch maintenance, Vibrator bowl vibration assembling coil/ strips out of order. Ampoules/ Vials breakage during filling and readjustment of the machine by operator if it requires. All stoppered vials/sealed ampoules/containers shall be incubated as population 1 where in case of production the same is followed. If anything discarded during routine production for maintenance the same shall be incubated as population 2.

- Line stoppage for not less than a defined duration (to be defined as per micro stoppage expectations e.g. 5 to 10 minutes) without removing containers from the line.

- Removal of plastic bottles without insert / screw cap, which is to be discarded from the rejection track and simulate removal of plastic bottle jam in walking beam.

- Vials jam between rubber stopper machine and sealing unit.

- Intervention for empty/ filled container breakage on the conveyor belt or conveying device and remove the surrounding containers affected by breakage.

- If any non-viable particle excursion observed in grade-A and/or Grade B area during execution of media fill, then the same and related actions as per SOP shall be considered as non-routine intervention and recorded in media fill BMR.

- Monitoring of non-viable count using off line particle counters.

- Monitoring of viable monitoring through offline active air sampling.

- Simulation of full door opening of filling room

33 Type, Frequency and duration of Interventions

- The company may consider to include the intervention at a higher than normal frequency/ duration in the APS. The frequency of inherent interventions during the APS is generally consistent with the frequency during routine production. The duration of the APS should be long enough to capture the potential microbiological impact of performing those interventions. Corrective interventions should be performed at a frequency, duration as defined in the aseptic process simulation protocol.

- Type, frequency and duration of interventions should be defined in APS protocol based on commercial batches data trending. This should be determined as defined as discussed previously in this guidance and specimen attached as Appendix II can be followed for the same.

34 Sterilization of contact parts

- The components like product contact parts such as Vials, PFS, Cartridge, Containers, rubber stopper etc. should be sterilized using validated sterilization cycles and load pattern.

- Validation studies should be conducted to demonstrate the efficacy of the sterilization cycle. Requalification studies should also be performed on a periodic basis. Media fill to use same validated loading patterns, critical parameters e.g. speed, temperature, time, pressure etc. as is used/to be used in routine production/process for which the simulation is planned/to be done.

35 Leak Test

- The processes to be simulated are defined as any and all manufacturing steps which occur after product equipment and container/closure sterilization and can adversely affect the sterility assurance of the product.

- In some cases, this may include process/ handling steps subsequent to sealing of the container (e.g., leak detection, automated inspection, etc.) where damage from handling can adversely affect product container integrity.

- Any subsequent inspection or handling steps, which may affect sterility assurance of the product should be part of APS.

- Perform leak testing of all containers (population 1) as per quantity mentioned in BMR as per respective leak testing SOP. Leak test passed containers shall be subjected to incubation.

- Leak test shall be performed as per actual leak test procedure for concerned container closure system with respect to routine production process, as applicable.

- If the Leak testing is the part of aseptic processes of the product batch, then Leak test shall be performed for the media filled containers.

- If any container found as leak test failure and observed as obvious non-integral based on the visual verification, then it should not be incubated and recorded as non-integral in rejection section, whereas the container found as leak test failure and observed as integral based on visual verification, then it should be proceeded for incubation as pollution -2.

36 Visual Inspection (Prior to Incubation)

- The normal product inspection process is qualified for the removal of non-integral containers (e.g., missing or misaligned closures, cracks in glass, poor crimps, etc.) due to the possible breach of product sterility. This inspection process should be maintained for APS filled units, with non-integral APS units removed during the pre-incubation inspection. Sort out and reject those containers having obvious breach of container/ closure integrity such as cracked container, broken container (visually leaking), containers with missing rubber stopper /closures. The rejections are to be discarded after these are identified for obvious breach of container closure integrity and to be recorded. The removal of such non-integral units is appropriate as failure to do so can lead to false-positives that may inaccurately represent the sterility control of normal operations.

- For the purpose of the APS, Container found to have defects (i.e. cosmetic, particulate and fill volume defects) should be ignored not related to integrity should be incubated; containers that lack integrity (leaking/open/un-sealed/un-capped) should be rejected.

- Erroneously rejected containers should be returned promptly for incubation with the media fill lot after Quality assurance physical verification and authorization.

- Personnel/ operator involved in the inspection of the media fill, they should have appropriate education, training, and experience in inspecting media fill units for microbiological contamination. The inspection to be performed by qualified inspector for detecting any type of contamination, the inspection to be with oversight by another qualified personnel from microbiology and/or quality unit.

- Visual inspection of media fill containers to be performed by use of a visual inspection booth against the black and white background, having lux level between about 2000 lux - 3750 lux.

- Gentle swirl and Invert of the containers to be done prior to incubation to allow media to contact with the inner surface of the containers and closure.

- After the visual inspection of “prior incubation stage” is completed for the media fill units, the required number of filled media fill units to be checked for the yield limit requirements for units to be incubated as defined in respective protocol of media fill.

- After 7 days (NLT 168 hours) and also after 14 days (NLT 336 hours) of incubation, inspection of all containers for Turbidity / color change to be performed. During this inspection, gentle swirl and inverting of the containers again will allow media to contact with the inner surface of the containers and closure thus ensure an opportunity for potential microorganism growth / recovery from all contact surfaces if any.

- In case of color indicator media, containers with suspected microbial contamination can be identified by color change from pink color to yellow color. Hence opening of media fill containers and transfer of medium in transparent containers is not required.

- In case of semitransparent containers (i.e. plastic bottles) filled with color indicator medium, visual inspection shall be carried out with same manner as other containers after 7th and 14th day of incubation.

- If the turbid container is failing in the Leak testing, then it should be addressed through impact assessment of the same on media fill. Based on the impact assessment, validity of the media fill should be concluded by Quality unit.

- As mentioned earlier, visual inspection shall be done by qualified visual inspector under the supervision of quality person/ microbiologist

37 Time restrictions and media fill incubation

- Filled APS units should be incubated without unnecessary delay to achieve the best possible recovery of potential contamination. A time limit to be defined from end of filling to incubation to avoid any delay in recovery/growth of organism. During these period the container are to the stored between 20-25 ° C till start of incubation.

- It is generally accepted to incubate at 20-25° C for a minimum of 7 days (NLT 168 hours) followed immediately, or after a first reading, by incubation at 30-35° C for another 7 days (NLT 168 hours), as a total minimum incubation time of 14 days. Starting with the lower temperature to avoid recovery challenge to any organism which grows suitably at low temperature.

- As mentioned above, filled APS units should be inverted or manipulated to ensure contact of the medium with all internal surfaces of the closure system before they are incubated. Units having cosmetic defects or those which have gone through non-destructive in process control checks should be identified and incubated. Non-integral units discarded during the process simulation and not incubated to be compared with units discarded during a routine production fill. If there higher discard in media fill, then the same to be investigated.

- Where processes have materials that contact the product contact surfaces but are then discarded, the discarded material should be simulated with nutrient media and be incubated as part of the APS (e.g. initial purging of filling set us, sampling of sterile solution from sterile suspension tank/sterile bulk if any) as population 2.

- Filled APS units should be incubated in a clear container to ensure visual detection of microbial growth. Where the product container is not clear (i.e. amber glass, opaque plastic), clear containers of identical configuration may be substituted to aid in the detection of contamination. When a clear container of identical configuration cannot be substituted, a suitable method for the detection of microbial growth should be developed and validated.

38 Media Fill Reconciliations

- As the target acceptance criterion for an aseptic process simulation study is NIL contaminated units, a high level of APS unit control and accountability is necessary. Accurate counts should be performed at each step in the simulation e.g. filling, pre-incubation inspection, and post-incubation inspection. At the conclusion of the post-incubation inspection, filled units are re-counted to verify pre-incubation accountability. In the event of a discrepancy an investigation should be performed to determine the source of the variance and potential impact on the validity of the APS study.

39 Visual Inspectors Qualification for Media Fill

- The firm has to define the procedure for qualification and requalification of the media fill visual inspectors and include the frequency for requalification of these qualified inspectors.

    - Good containers as well as physically defected rejections of the all type of containers shall be preserved from the previously executed media fill for preparation of media fill visual inspection kit and media fill library.

    - QC microbiology has to inoculate the different containers filled with media using representative microorganisms (Gram positive, Gram negative, mold and yeast).

- Inoculum should have strength about 10-100 CFU.
- All such containers should be incubated as defined in below table for bacteria and fungi.
- In some cased McFarland standard (these are standards used as reference to adjust turbidity of bacterial suspension) can be used for Visual Inspector qualification. If it is used, then volume shall be filled with McFarland standard to be same/similar as used for media fill and routine production.
- During Visual inspector qualification, collect the good containers, rejected/ defected container and turbid containers if any.
- However, total number of the containers should be taken at-least sufficient for one session of visual inspection prior to eye rest as per SOP. E.g. If after every one hour there is eye rest of 10 minutes, then total quantity for inspector qualification to be equal to quantities that need to be inspected in 1 hour. If one unit takes 15 seconds for inspection, then 240 units to be taken in the challenge kit for one hour inspection. The inspector has to complete inspection of these 240 units within 1 hour during qualification study.
- Total container should be taken in such way that all type of defected and turbid container to covered and challenged in the test kit.
- Defected (integral and non-integral) and turbid containers should be not be more than 1% of the good container used for qualification of the visual inspectors.
- For qualification of Visual inspector, containers (Good, Defected and turbid+ color change) should be provided to visual inspector for its identification.
- Acceptance criteria: Visual inspector should have to identify all (with 100% accuracy) the defective and turbid containers from the challenging test kit.

40 Growth promotions test (GPT) of Media (Standard + EM Isolates)

- Media used for the media fill simulation study should pass for the growth promotion by use of pharmacopeial method. GPT is an essential control for media fills because the desired test result is “NIL growth” and only by demonstrating the medium’s ability to support microbial growth will confirm that there is no false negative.
- Growth promotion testing of the media used in simulation studies should be carried out on completion of the incubation period (after 14 days incubation) to demonstrate the ability of the media to sustain growth, if contamination is present. Growth should be demonstrated within 5 days at the same incubation temperature as used during the simulation test performance. (Refer below table for reference).

- Media should be evaluated for growth promotion properties by challenging the standard pharmacopeial microorganisms (Gram positive and gram negative bacterial, yeast and mold) and Environment isolates isolated from the environment and/ or from the sterility test positives if any.

GPT Incubation conditions			
Challenged Microorganisms And specified strains*	**Incubation Temperature**	**Incubation time**	**Inoculum strength**
Staphylococcus aureus Such as ATCC 6538, NCIMB 9518, CIP 4.83, or NBRC 13276	30°C to 35°C	≤ 3 Days	≤ 100 CFU
Pseudomonas aeruginosa such as ATCC 9027, NCIMB 8626, CIP 82.118, or NBRC 13275	30°C to 35°C	≤ 3 Days	≤ 100 CFU
Bacillus subtilis such as ATCC 6633, NCIMB 8054, CIP 52.62, or NBRC 3134	30°C to 35°C	≤ 3 Days	≤ 100 CFU
Candida albicans such as ATCC 10231, NCPF 3179, IP 48.72, or NBRC 1594	20°C to 25°C or 30°C to 35°C	≤ 5 Days	≤ 100 CFU
Aspergillus brasiliensis such as ATCC 16404, IMI 149007, IP 1431.83, or NBRC 9455	20°C to 25°C or 30°C to 35°C	≤ 5 Days	≤ 100 CFU
Environment in-house Isolates# Isolated from production environment and/ or positive sterility test.	20°C to 25°C or 30°C to 35°C	≤ 3 Days or ≤ 5 Days	≤ 100 CFU

- Note: *: Challenged microorganisms can be used as equivalent strain as specified in above table.

- #: Incubation Temperature and time can be used based on the type of environment Isolates e.g. If the Environment isolate is bacteria then incubation condition can choose as 30° C to 35° C for ≤ 3 Days, Whereas Environment isolate is fungi then incubation condition as, 20° C to 25° C for ≤ 5 Days. Environment isolates shall be based on the evaluation of the data(s) for re-occurrence of the Environment in-house isolates. The guidelines generally require that referenced microorganisms from the Pharmacopoeia are used. Besides this, isolates from monitoring are covered.

Overview of Requirements for Referenced Microorganisms in Checking of Growth Promotion:-

Guidance for referenced microorganism for GPT		
Guideline	**Description**	**Microorganisms**
FDA Guidance on Sterile Drug Products Produced by Aseptic Processing	In this regard, it is valuable to incubate positive control units along with media fill.	❖ Microorganisms referenced in USP 7 growth promotion tests ❖ Types of microorganisms that have been identified by environmental monitoring (regularly - every 6 months - updating is necessary) ❖ Types of microorganisms that have been identified by positive sterility test results (caution because of the probability of secondary contamination)
FDA Guidance on Sterile Drug Products Produced by Aseptic Processing	When performing a process simulation run, it is valuable to incubate positive control units inoculated with < 100 CFU challenge.	
USP <1116> Microbiological evaluation of Clean Rooms and Other Controlled Environment	In general, an all-purpose, rich medium such as Soybean Casein Broth that has been checked for growth promotion with a battery of indicator organisms at a level of below 100 CFU / unit, can be used.	❖ Indicator microorganisms 7 ❖ Isolates from the controlled environment where aseptic processing is to be conducted may also be used (regularly - every 6 months - updating is necessary)
Recommendation on the validation of Aseptic Processes, PIC/S PE 002-1	Growth promotion tests should demonstrate that the medium supports recovery and growth of low numbers of microorganisms, i.e. 10 - 100 CFU/unit or less. Growth promoting testing of the media used in simulation studies should be carried out on completion of the incubation period to demonstrate the ability of the media to sustain growth if contamination is present. Growth should be demonstrated within 5 days at the incubation temperature as used during the simulation test performance.	❖ Bacillus subtilis ❖ Staphylococcus aureus ❖ Candida albicans • ❖ Aspergillus niger/ ***brasiliensis*** ❖ Clostridium sporogenes (process simulation with thioglycollate medium) ❖ In-house flora (e.g. isolates from monitoring etc.: regularly - every 6 month - updating is necessary).

PDA-Technical Report No. 28	Confirmation of the media's growth promotion properties is an essential element ... The growth promotion units should be inoculated with a low concentration (less than 100 organisms per container) ... Media growth promotion studies can be performed prior to, concurrent with or after the completion of the process simulation incubation period	❖ The USP growth promotion organisms Bacillus subtilis & Candida albicans. (It makes more sense to use the whole range of microorganisms referenced in the Pharmacopoeias than to choose only two representatives). ❖ Other organisms commonly found in the aseptic processing area environment such as organisms isolated during personnel monitoring (regularly - every 6 month - updating is necessary), sterility testing (caution because of the probability of secondary contamination), etc.
Aseptic Processing of Health care products - Part 1: General requirements, ISO 13408-1	Verification of growth promotion of media used in specific media-fill runs shall be conducted following the run	Test organisms in conformance with pharmacopoeial requirements.

- Note: A "ready to use" liquid medium supplied from a commercial vendor should be confirmed as suitable by growth promotion and other testing to confirm that it meets specifications and conforms to its CoA. Once a supplier has been demonstrated to provide consistently suitable medium, the CoA and positive control will suffice to establish the medium's suitability for use in media fills (the positive control will be used in lieu of demonstrating growth promotion potential).

- Growth promotion testing confirms the medium's ability to support growth. Growth promotion testing is commonly done before using the medium in an experiment. A positive control tests the ability of the test method to result in a positive outcome and is commonly done concurrently with an experiment. Media fill positive control shows that the medium in the drug product container will support growth after exposure to the filling process. The positive control should be a container filled as part of a media fill.

- The positive control test may serve as the growth promotion test for the medium, as long as a qualified vendor is being used.

    - A positive control is needed for each media fill that is performed using a single batch of medium. As stated previously, a positive control in the media fill may also serve as the growth promotion test of the medium (from qualified vendor) employed for the media fill.

    - When performing a media fill, the positive control test may be done simultaneously with the media fill by inoculating a vial of medium from the same batch used for the media fill.

    - Preferably a positive control perform at the end (after incubation) of a media fill by inoculating an uncontaminated media fill test container and returning it for additional incubation. To evaluate the media is still able to support growth even after completion of the incubation at provided temperatures. So if any microbial contamination in the vial, can be cultivated and promoted for growth during the incubation.

    - Inoculation of the positive control container should be done in an area separate from the critical manufacturing area.

41 Use of Color vs. Conventional Media

- The media fill can be performed using either conventional media or media with color indicator. Both media having growth promotion properties as desired for media fill.

- Manual reading of several thousands of pharmaceutical containers filled with culture media is the current practice. Since each container must be individually checked, this step is extremely time-consuming and can be a source of potential errors. It requires highly qualified technicians/personnel and time. The same to be performed in appropriate conditions as well.

- A culture medium for media fill with a color indicator optimizes and facilitates the reading step and can lead to less false reading errors.

- The color indicating media formulation was designed to meet the highest requirements from the pharmaceutical industry. Irradiated, cold filterable and animal free, this vegetable TSB was also optimized for the growth of anaerobic micro-organisms to reach the highest level of performances.

- Color indicator Media contents vegetable peptones instead of animal-derived peptones for “animal-free” facilities. Besides, considering the importance and the sensitivity of the media fill.

- This vegetable formulation a growth-based proprietary color indicator to help for the reading of contaminated units. Contamination and turbidity can easily be revealed with an irreversible color change if growth occurs after incubation.

- The media support the growth of all type of microorganisms such as Aerobic, Anaerobic, Mold, fungi etc.

- Thus, the media is soluble in nature for easy to handle using media preparation and it can be filtered through the 0.22 μ filters.

- Considering the above, it is recommended to use the sterile color indicator media for avoid error during inspection and fasten the process and quick identification of the positive containers, if any.

- Color indicator media shall be verified and validated to check the color change and/ or turbidity by inoculating the challenges microorganisms (10-100 CFU), including the environment isolated. Hence to check whether the color is change in media in present of the microbial growth.

- The dehydrate media commercially available as sterile by validated Gamma sterilization method. Media is evaluate for sterility assurance and growth promotion properties after the gamma sterilization.

- Sterile media will increase the self-life and avoid adulteration of the media, as it is free from the microbial contaminations.

- Color indicating media is very essential for translucent plastic containers and smaller container, and/or containers with narrow lumen to avoid error. E.g. plastic ophthalmic products container, blow fill seal containers, pre-filled syringe, small vials, cartridges etc.

42 Acceptance criteria

- The ultimate goal for the number of positives in any process simulation should be NIL.

- This is true regardless of the number of units filled during the APS or the number of positives allowed. A sterile product is by definition, one which contains no viable organisms.

- Regulatory authorities have provided guidance on process simulation acceptance criteria and these should be well understood before developing internal requirements. However, there are numerous technical problems in achieving this goal. For example, media and simulated product do not completely mimic real products in terms of their processing characteristics and microbiological growth supporting properties. There may be differences in solubility, pH, filtration rates and filterability and viscosity. With powdered products, the process simulation involves reconstituting powdered media or simulated product, introducing extra processing equipment or manipulation, with the inherent risk of contamination. Since a microbiological medium is designed specifically to support or stimulate the growth of microorganisms, it is a more rigorous challenge than processed products, which often provide neutral and sometimes hostile microbial growth environments.

- For these reasons acceptance criteria with a limit of some low number of positives, other than zero, are often chosen, consistent with applicable regulatory requirements.

- However, the target should be zero growth any positive to be investigated.

    - Any positive unit is significant, regardless of run size, and should result in a thorough, documented investigation. Following the investigation, appropriate corrective action may be taken based on scientific evaluation and risk assessment.

    - Process simulation contamination rates approaching zero should be achievable using well designed and controlled aseptic filling operations, especially those involving automated production lines in well-designed aseptic processing facilities, blow-fill-seal / form-fill-seal and in isolator/barrier based systems with design, process control with adequate qualified, trained personnel with appropriate understanding and attitude for aseptic sterile operations, patients safety.

    - In any condition is contamination is more than one, then after root cause finding and implementation of corrective action, a revalidation of media fill to be planned. It should be with 3 consecutive successful media run.

43 Container closure Integrity Testing on Media Fill

- The integrity of particular container/closure configurations should be assured by:
    - Media fill containers can be used after 14 days of incubation completion from successful media fill run. Validation of the closure system to be done by inserting these container in a broth containing approx. 106 cfu/ml of a suitable micro-organism by use of both pressure and vacuum. The media fill containers are to be removed after submersion for a recognized period of time, disinfected and then incubated for 14 days. Growth in media inside the container would indicate a failure of the closure system.
    - The container/closure integrity test is normally checked independent of media fill. The capping/sealing machine set-up is however a critical factor. For such containers, the set-up of the capping machine may be critical as the operation can cause distortion of the stopper if the capping force is not adequately controlled. The validated parameters of machine to be used for container closure integrity (capping) and same to be there for media fill.
    - The container closure integrity validation should take into consideration any transportation or shipping requirements that may negatively impact the integrity of the container (e.g. by decompression, pressure etc.)

44 Media Fill abort

- Media Fills can be aborted only for the reasons extrinsic to the process. As with any aseptic process validation run, it is important to note that invalidation of media fill run should be a rare occurrence. A media fill run should be aborted only under circumstances in which written procedure require commercial lots to be equally handled. Supporting documentation and justification should be provided in such case.
- Aborted media fill shall be investigated and appropriate corrective and preventive action have been initiated.
- Though not limited, in following circumstances, media fill run shall be considered as aborted and as the same applicable for aseptic routine process as well :
    - Major machine break down (e.g., Major maintenance on Filling and stoppering machine)
    - Major Power failure (AHU / LAF Stoppage)
    - Leakage and/ or damage in the filling assembly, sterile bulk containing line, tube and/or tank
    - Any atypical event which could have direct impact on the sterility assurance
- Media fill abort should be pre-approved by the quality unit based on the impact assessment and review.

45 Invalidated Media Fill

- It is important to note that invalidation of media fill run should be a rare occurrence.
    - In following circumstances, media fill run shall be considered as invalidated:
    - The Positive control (Growth promotion test) is failed in the executed media fill.
    - Incubation condition of the media fill containers not maintained as defined.
    - In the simulation of a Lyophilization process, the depth of vacuum drawn on the chamber and the period of time for which this vacuum is held are important considerations. If the medium in the container to boil out due to the Vacuum level maintained in the chamber, thereby invalidating the simulation.
    - The organization has to define the conditions that may cause the simulations to be invalidated.

46 Media Fill Failure Investigations

- Media Fill Failure Investigations
- The target should be zero growth. Any contaminated unit should result in a failed process simulation and the following actions should occur for investigation.
- It should be documented/logged in quality management system document, management to be notified through a quality alert and/or field alert report (FAR) as applicable e.g. as un-planned deviation and/or firm's procedure for such incident logging to be followed.
- Cross function investigating team to be formed with subject matter expert from microbiology, quality assurance, engineering, operations and if required from any other cross function team.
- Investigation plan to be prepared as per each firm's SOP for such investigation.
- Identification of organism to be initiated. If there is gross contamination and/or contamination found in several containers, then a sound scientific rationale to be prepared for identification of containers e.g. from each tray, initial, middle and end tray in which contaminations are found, based on AQL etc.
- If contamination were observed before completion of full incubation period, then few similar looking contaminated containers to be taken for identification of organism while balance contaminated container and the good containers are to be continued with incubation to complete total 14 days of incubation.

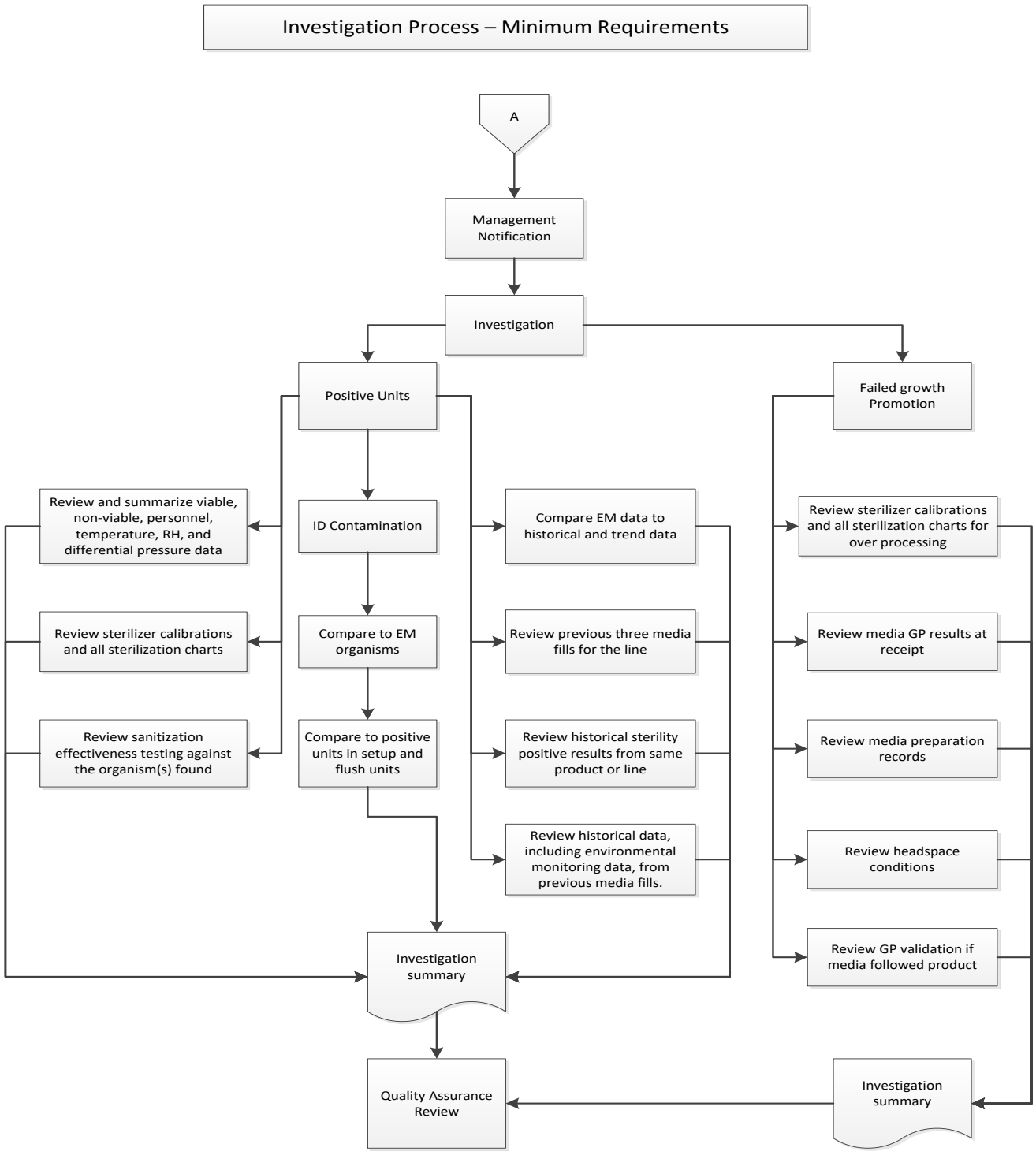
- Though not limited, the investigation to done as per process flow and probability of contamination at each process step to be evaluated. The below review should also to be part of investigation.
    - Document/batch record review
    - Equipment review including HEPA filter certification status of room(s) involved, airflow pattern of room(s) involved and storage condition for sterile components and equipment prior to use
    - Actual process intervention review from media fill video and also comparing that with qualified air flow pattern
    - Environmental parameters review for pressure differential, viable, non-viable particle count, temperature, humidity, personnel monitoring data etc.
    - Material review including water used to manufacture the nutritive media and ready to use component sterilization validation data e.g. sterility of lactose or any material required for simulation of sterile material addition
    - A historical time sequence of activities leading up to the failed unit
    - Review of cleaning and sanitization records
    - Review of manufacturing process
    - Review of Filtration process including pre and post integrity test results for sterilizing filters and tank filters/utility filters
    - Review of Filling process
    - Review of Lyophilizer Loading and unloading
    - Review of Sterilization data (e.g. Autoclaves, Depyrogenation tunnel, sterilization in place etc.)
    - Review of process event from equipment, alarm records of equipment used for aseptic process and sterilization etc.
    - Review of Primary packing materials
    - Review of Interventions (Routine intervention/ Non Routine interventions) including any unusual occurrence during the media run.
    - Any new/existing worst case simulation and its impact if any
    - Review of Change control record/ Qualification/ Validation/ Calibration/ Maintenance / Preventive Maintenance since last successful media fill for aseptic process
    - Review of facility

- For new facility and/line, review of design with respect to sterility assurance
- Review of impact assessment (Detailed impact assessment shall include following, but not limited to)
    - Review of environmental monitoring data
    - Review of personnel monitoring data
    - Review of Bulk and Finished product sterility results
    - Review of sterility failures
    - Review of market complaints related to sterility if any
    - Review of control sample inspection results
    - Review of stability study results with respect to sterility and container closure integrity
    - Review of event or Deviations related to sterility assurance
    - Review of QA oversight observations related to sterility assurance
    - Review of Facility or equipment failure Deviations
    - Review of Periodic requalification of sterilizing equipment
- Review the historical and trend data for a minimum of 25 such previous environmental monitoring sessions for rooms where the media fill study occurred, and the remainder of the aseptic area.
- Review and discuss the historical data, including environmental monitoring data, from previous media fills. Minimally review and discuss the previous three media fill for the same line.
- Review of Media fill Incubation data
- Review of Visual inspection data of different stages
- Review of Hold time data
- Review of Personnel involved in the media fill operation (Training, Qualification and attitude)
- Review of Stage wise rejection percentage for non-integral containers with respect to its limit
- Review of Growth promotions test
- Review of Leak testing data prior to incubation
- Review of Leak testing data of turbid containers

- Microbial Identification of the contamination and source of contamination
- Review and discuss the historical sterility positive results from the same product or filling line since the last successful media simulation
- Review of video records (CD/ DVD etc.)
- Review of the observations during execution of the media fill activity
- Review and discuss results of any available sanitization effectiveness testing against the organism(s) found

- Most probable / root cause determination
- Determination and implementation of appropriate corrective measures.
- A sufficient number of successful, consecutive repeat media fills (normally a minimum of 3 consecutive media fill) should be conducted in order to demonstrate that the process has been returned to a state of control.
- All products that have been manufactured on a line subsequent to a process simulation failure should be quarantined until a successful impact assessment performed.
- Production should resume only after completion of successful revalidation.
- Note: If a media fill passes/successful for an aseptic process / line, it does not mean all batches to be produced on same line will have full sterility assurance. Sterility assurance depends on various factors e.g. experience, qualification, attitude of people, facility, equipment design, actual practice for particular batch, environmental data, maintenance, changes if any.

Media fill Investigation flow chart



47 Media fill Video recordings & its review

- The firm has to define the procedure for video recording during the media fill (APS) and its review.

- Video recording" should be performed during media fill. Video shooting to capture all the activities from point of sterilization onward during each media fill simulation process. It should be done from outside or through a CCTV camera with full visibility of aseptic process. Where required, video recording should be used as an aid for investigation in case of any unit showing growth to investigate and to assist with identifying the root cause.

- It is recommended to review each media fill batch/ run to conclude the media fill simulation. Video recording of the media fill run (APS) shall be reviewed to determine the expected corrective action and refreshment training requirement for the personnel. Reviewer should be trained and qualified on the media fill program and basic microbiology. Reviewer has to identify an inadequate practices/ behavior in aseptic techniques having potential risk on the sterility assurance and are of improvement in term of intervention handling, Line set up and further automization. Reviewer may be from cross function team and/or from quality unit.

- Video recording review shall be documented. If observations identified during this review shall be recorded, assessed and if require investigation to be performed. Storage period of media fill video should be same as media fill batch record / protocol.

48 Personnel Qualifications (Activity Based)

- Each person in the aseptic filling area (e.g., operations, engineering, and quality) has the potential to introduce microbiological contamination; however, the risk to product may vary with the specific job function.

- Personnel working in the clean room should be capable of adequately performing their job function, properly trained in their work function, and qualified to perform those functions. Work functions include aseptic process gowning, clean room practice, aseptic technique, as well as specific operational functions. Operational functions may include filtration and fill system set-up, adjustment, repair, maintenance, cleaning, sanitization, operation, component and product handling, transfer, sampling, monitoring, oversight, supervision and other inherent and corrective interventions.

- The requirements for the qualification of clean room personnel should be written in a formal procedure and the results are to be documented.

49 Personnel Prerequisites

- Personnel must successfully meet the firm's gowning certification requirements. They should have completed all relevant training, including but not limited to cGMP training, procedure specific training, gowning training, clean room practices training, training in basic microbiology and specific clean room operation, function and relevant intervention procedure training.

- Aseptic technique of personnel like operators, micro biologists, Engineer, EM operators, QA personnel, supervisor that works in the filling room must be simulated at least once per year by participating in a media fill for the activity performed by each individual. Personnel participating in the media fill should perform routine activities/ similar type of function for that process.

- Participation should be consistent with the nature of each operator's duties during routine production for which the person is qualified as defined in personnel qualification matrix

A. Initial Qualification

- Personnel should demonstrate their skill in aseptic technique by successfully performing a qualification test, require successful manual manipulation not associated with media fill in a mock trial and / or on placebos, water trials.

AND

- Participation in a successful aseptic process simulation run in which personnel perform the same function (s) [Critical operation such as Line set up (Assembly), filling, interventions, filtration, FD loading and unloading, supporting activities during line set up, Break down maintenance, Environment Monitoring, sampling etc.]

B. Periodic Qualification

- Personnel should participate in a successful Media fill (APS) run in which they perform the same function(s) to the extent that they will perform it during actual production at least once per year.

- Personnel should have to complete his refreshment training or verification of the training on the latest version of the Aseptic operational procedures, followed by extensive gowning samples for microbiological evaluation.

C. Dis-qualification

- Previously qualified personnel may be considered to have lost that qualified status (Disqualified) status if one or more of the following occurs:
    - They failed to participate or present in the media fill, once in a year to simulate the same function / activities perform in routine production.
    - They participate in a failed media fill, where the cause of the failure is related to their performance.
    - They perform in the clean room or the workplace in a manner supposed unacceptable in relation to clean room or aseptic process operations or functions/ Aseptic behavior.
    - They fail to maintain gowning certification (failed to participate in periodic qualification gowning sampling).
- The individual's qualification can be re-established once the specific deficiency is properly remedied.

D. Access without prior qualification

- It is recommended that the firm to have a procedure for non-qualified personnel entry such as:
- There may be situations where non-filling personnel must enter into an aseptic processing area during an aseptic process to observe or perform non-aseptic process activities. It is recommended that individuals who have not successfully completed qualification be closely supervised and accompanied, while in the clean room/ aseptic area by the qualified personnel. These personnel should not be present during critical aseptic process steps. Their access to the aseptic processing area be restricted to the specific function required (e.g., equipment maintenance, audit, etc.). It is recommended that non-qualified personnel should be verified for health and hygiene and trained minimum on the entry and exit gowning procedure and do and don't on the Aseptic practices.

50 Engineering Media Fill (if required)

- If new facility/ new line and technology like Isolator, RABS are newly introduced, then it is recommended to perform the engineering run/ placebo run for identification interventions, type of intervention, frequency, duration and constraint (if any) during operation of the aseptic process and equipment operation.

- The use of engineering runs to develop the process is strongly encouraged. Engineering media fill in the new facility and new line to evaluate the scope for improvement in the state of the control of the defined aseptic processes, before proceeding for the aseptic process simulation / validation.
- Engineering media fill run supports to take further decision for further validation and expected corrective action if any.

- Engineering media fill should be documents and reviewed by the quality unit. If any failure then investigation shall be performed for root cause identification and appropriate CAPA to be implemented.

51 Deviations in Media Fill

- Any deviation observed during Aseptic process simulation (Media fill), then it should be investigated and documented in the summary report. The firm has to define/ establish the procedure for investigation, impact assessment, root cause analysis procedure and CAPA initiation. Each deviation should be timely reported and reviewed by quality unit.

52 Do's & Don't of Media Fill

- All the routine interventions must be conducted at more or at least in similar intervals as in normal production.

- All interventions, including non-routine interventions must be documented properly in media fill protocol along with type, frequency and duration.

- Interventions should not be designed or selected to justify poor process or facility design or to assess unacceptable interventions that rarely occur and which should lead to a thorough investigation and product assessment when they do occur.

- Each tray after capping/ Sealing shall be labeled with media fill no., tray number, Date, time, run number. If any media fill failure occurs, then the turbid container shall be reconcilable with the tray/run no., approximate time and the activity being simulated during the media fill.

- Media fill container fallen on the floor prior to capping/sealing and inside FD chamber to be considered as rejection and shall be discarded after completion of batch.

- Only those containers that have an obvious breach of container closure integrity such as cracked/ broken container (with visible leaking if any), containers with missing rubber stopper/ closure and Filled unstopped/ half stoppered fallen container shall be rejected.

- The filling rejection only is discarded after assignable cause identified for obvious breach of container closure integrity and QA authorization.

- Media should not be frozen at any stage during simulation of Lyophilization cycle in case of freeze-dried products.

- The release of the vacuum must be done with a gas that shall not inhibit aerobic micro-organisms growth. For Example sterile compressed air shall be substituted for sterile nitrogen gas in case of lyophilized products.

- "Video recording" should be performed during the process simulation using media fill.

- Media fill reconciliation documentation shall include an accurate accounting of filled and sealed containers and type of non-integral defects rejected from the media fill.

- At the conclusion of the post-incubation inspection, filled units shall be re-counted to verify pre-incubation accountability.

- Non-representative, extreme, artificial environmental conditions shall not be simulated.

- Interventions that would represent an unreasonable risk of contamination should not be included in either process simulation or routine production.

53 Post Media Fill Cleaning and Sterilization of Equipment

- The firm has to define the procedure for extensive cleaning and sterilization of the equipment at post media fill. Cleaning and sterilization of the equipment shall be performed after each media fill Batch.

- Equipment used for manufacturing, filtration and filling to be cleaned by cleaning solution as per validated cleaning procedure.

- Product / Media contact parts must be cleaned as validated cleaning procedure and should be sterilized within cleaning hold time. It is recommended that equipment shall be cleaned and sterilized twice before use for routine production.

54 Decontaminations post to Media Fill

- Sometime, there is a concern over the possible contamination of the facility and equipment with nutrient media during media fill runs. However, if the medium is handled properly and is promptly followed by the cleaning, sanitizing, and, where necessary, sterilization of equipment, subsequently processed products are not likely to be compromised.

- Cleaning and sanitization of the manufacturing, filtration and filling area, furniture, etc. by cleaning/ sanitizing solutions followed by sporicidal solution within stipulated times.

55 Handling of Media Spillage

- Media fill simulations in which chances of media spillages are there, a proper decontaminating agent shall be used for media decontamination. It shall be treated as potential contamination spillage and shall be handled as per cleaning and sanitization process of respective area/equipment etc.

56 Destruction of Media Fill Containers and Residuals

- Dispose the media filled container after approval of Quality assurance. Crush the media filled container in crushing machine. Sanitize crushing machine and area with approved sporicidal solution. Discard the crushed containers after adding sporicidal solution in to the container containing crushed containers. Media collected in the plastic containers for disposed as per defined procedure.

- Contaminated containers of the media fill shall be decontaminated by sterilization before its disposal and destruction.

57 Myths

- Myth1: If any Major Modification in non-sterile area (Manufacturing area- Grade C and D area) is there, then it requires media fill.
    - Media fill is not required, if it has no impact on differential pressure and area integrity of the Aseptic area.

- Is schedule Media fill (APS) failure, required recall for commercial batches, since last pass/successful media fill batch?

- The aseptic process simulation (Media fill) batch pass or failure is indication, only a demonstration of the capability of the process to produce sterile products aseptically at the time of its execution (that particular event and batch) using the defined process, materials, facility, equipment and personnel. The APS does not provide information which relates directly to the sterility of a specific product batch. Therefore, the fact is that, a specific APS does not meet the required acceptance criteria does not necessarily indicate a sterility problem for any particular production batch. However it is an indication that some event has occurred during the APS leading to contamination of one or more units.
- If there is no systemic failure and deficiency identified as root cause of the APS failure, then impact on distributed batches to be determined.

- High-risk aseptic intervention, technique or practice is simulated in APS, can justify in use or acceptable during production?
  - No, High risk aseptic intervention, technique or practice is not acceptable and can't justify to use during production of the commercial batches.

- Each Hold times shall be simulated in all media fill runs (APS).
  - Hold times of each components, shall not be simulated in each media fill run, the hold times of sterile equipment, sterile closures and filtered media shall be covered under worst case matrix and to be validated initially/ rotational basis as part of media fills. Preferably single hold time shall be performed initially in each run instead clubbing of hold time in same run.

- Media fill is not required for each products
  - The Aseptic process simulation (media fill) is not based on the different products (Product name), but it should be based on the container closure systems (CCS) and its process and interventions. Bracketing or matrix approach may considered for validation of the same container/ closure configuration for different products where process equivalence is scientifically justified.
  - The new product introduction should be evaluated, based on the media fill matrix (Bracketing approach) and its processes, if the container closure system belongs to (in between) worst case container closure systems simulated in the media fill (APS), then Media fill is not require for the introduction of the new product. However if the CCS of the new product not fall in between the simulated worst cases container closure system, then media fill should be performed with new container closure system (Worst case CCS, either Higher or lowest), and APS Metric will be updated with revised new worst case CCS system.

- Leak test failure containers/ Cosmetic defected containers of APS should be incubated!
  - The APS containers, having cosmetic defect and failure in leak test, but the containers that haven't an obvious breach of container closure integrity [such as cracked/ broken container (with visible leaking if any)] container shall be incubated.
  - Whereas, only those containers that have an obvious breach of container closure integrity such as cracked/ broken container (with visible leaking if any), containers with missing rubber stopper/ closure and Filled unstopped/ half stoppered fallen container shall be rejected and not to proceed for incubation for evaluation of the APS.

- Operator qualified and simulated in APS for handling rubber stopper addition and assembly of the vial line, can't be considered for qualified for PFS line for handling of the Assembly and line set of the PFS line.
    - The Operator should be trained and qualified for gowning certification and has to participate to simulate his/her practice for function/ intervention on the process line and design. However the operator only participated and simulated in the same function in the vial processing line and design, he can't work for same function and intervention in different line/ design i.e. PFS line, which can't be justified the process and design similarity.

58 FAQ on Media Fill

- Can media fill exclude by bracketing approach, for introduction of the new Container closure system (CCS)?
    - Yes, if there is same design of the CCS (except dimension), aseptic processes, and intervention types, then only worst cases container closure systems (Max and min containers) should be simulated with 3 runs for each, while in between container closure system can be excluded.

- Whether HEPA filtration replacement in Grade B/ Grade A Area, requires media fill?
    - No, If HEPA filter replaced with same grade and size, then media fill is not required, however Air velocity and integrity to be checked before and after replacement filters, pre checked for old filters and post checked for new filters.

- Whether major modification in aseptic area requires media fill?
    - Yes, Media fill is required with risk based approach with any one and/or both worst cases container closure system. No. of runs to be decided based on risk evaluation.

- Can we justify the Pass media fill batch with Grade A EM excursion for Batch release?
    - No, if successful Media fill having excursion in grade A is found, then also we can't justify it for commercial batch release having grade A excursion. Batch disposition decision should be based on the impact assessment.

59 Appendix

- Appendix I : Risk Assessment

- Appendix II: Interventions trending and evaluation

60 References

- Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice; U.S. Food and Drug Administration: 2004 (Dec 8, 2011).
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- Technical Report No. 22 (Revised 2011): Process Simulation for Aseptically Filled Products
- Pharmaceutical inspection convention/ Pharmaceutical inspection co-operation scheme, Recommendation on the validation of processes (PIC/S, PI 007-6, 1 January 2011
- USP 43 <71> Pharmaceutical Sterility Testing; U.S. Pharmacopeia: 2013
- USP 43 <61> Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests; U.S. Pharmacopeia: 2013
- USP 43 〈1113〉 Microbial Characterization, Identification, and Strain Typing; U.S. Pharmacopeia: 2013
- USP 43 〈1116〉 Microbiological Control and Monitoring of Aseptic Processing Environments; U.S. Pharmacopeia: 2013

Appendix I
Risk Assessment of the Aseptic Filling Line

A. Suggested format and table for Assessment (FMEA)

Function / Sub-function / Process Step/Unit Operation	1.0 Man (Personnel)	Location/Area/Block :	Filling line Number
Reference Document Number / Type:	Unique number of reference procedure		
Risk Input (Potential failure Mode/Unwanted Event):	Human errors and risk of microbial contamination		
Causes:	❖ Inadequate training and qualification ❖ Inadequate aseptic technique ❖ Inadequate Personnel Hygiene and cleanliness ❖ Inadequate movement in aseptic area. ❖ In adequate personnel movement. ❖ Out of limit personnel monitoring results		

RISK ASSESSMENT & EVALUATION

	Severity* (S)	Occurrence* (O)	Existing Control for detection of potential failure	Detection* (D)	Risk Classification	Recommendation for mitigating risk	Responsibility & target completion Date
Rating	XX	XX	ABC	XX	XX	XX	DD/MM
Justification							

B. The potential risk and its failure/ cause(s)

Function / Sub-function / Process Step/Unit Operation	Risk Input (Potential failure Mode)	Cause(s)
1. Man (Personnel)	Human errors and risk of microbial contamination	❖ Inadequate training and qualification ❖ Inadequate aseptic technique ❖ Inadequate Personnel Hygiene and cleanliness ❖ Inadequate movement in aseptic area. ❖ In adequate personnel movement. ❖ Out of limit personnel monitoring results
2. Environment Control A. Facility Design	Impact on Environment	❖ Inadequate facility design for floor, wall, ceiling and surfaces. ❖ Inadequate pressure zoning ❖ Inadequate man and material flow ❖ Area classification is not carried out as per criticality of operation.
B. Decontamination, cleaning, sanitization	Impact on Environment	❖ Decontamination solution efficiency study is not performed. ❖ Criteria for selection of Decontamination solution is not available. ❖ Sanitization solution efficacy test is not performed. ❖ Schedule for rotation of sanitization solution is not in place. ❖ Sporicidal solution is not used in sanitization programme. ❖ Sanitization sequence is not define and followed, ❖ Multiple uses of sanitization aids. ❖ Drain sanitization frequency is not in place ❖ Preparation and storage controls are not in place. ❖ Inadequate Training of Personnel for cleaning and sanitization.
C. Temperature, Relative Humidity, Room Differential Pressure, LAF differential pressure	Impact on Environment	❖ HVAC failure, chilled water temperature higher. ❖ Dehumidifier malfunctioning. ❖ Door remains open / partial open. ❖ Reversal flow of Air ❖ Blower motor failure ❖ Damper position disturbed. ❖ In adequate door interlocking ❖ Mal-functioning of monitoring device ❖ Inadequate calibration & preventive maintenance
D. Non-viable particle count	Impact on Environment	❖ Inadequate sampling location selection. ❖ Frequency of monitoring not defined. ❖ Vibration / electric noise ❖ Inadequate cleaning of iso-kinetic probe and tubing. ❖ Sensor malfunctioning ❖ Inadequate EM operator training ❖ Inadequate handling of aseptic interventions. ❖ Sampling procedure not properly defined or followed. ❖ Calibration and preventive maintenance programme not in place.

Function / Sub-function / Process Step/Unit Operation	Risk Input (Potential failure Mode)	Cause(s)
E. Viable Monitoring	Impact on Environment	❖ Inadequate Sampling location selection ❖ Inadequate frequency of monitoring/frequency not followed. ❖ Inadequate sampling methods ❖ Inadequate cleaning and sanitization of areas ❖ Active Air sampler malfunctioning ❖ Inadequate Man-Material movement. ❖ Inadequate Aseptic area behavior ❖ Inadequate EM operator training ❖ Personnel monitoring programme not defined ❖ Anaerobic monitoring programme not defined ❖ Aseptic practices during Environment monitoring ❖ Media plate preparation and handling. ❖ Media plate transportation. ❖ Media plate incubation and inspection ❖ Calibration and preventive maintenance programme not in place.
2. Utility A. Pure Steam	Impact on sterilization process	❖ Breakdown of Pure steam generation system ❖ Inadequate input of source water and steam. ❖ High conductivity of pure steam ❖ Low pressure of pure steam ❖ Moisture content ❖ High non condensable gases ❖ High degree of superheat ❖ Instrument failure ❖ Operational errors ❖ Inadequate PLC controls ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.
B. Compressed air	Impact on product Quality	❖ Breakdown of Air compressor ❖ Low air pressure ❖ High water content ❖ High Oil content ❖ Instrument failure ❖ Operational errors ❖ Odor ❖ Failure Particulate matter ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.
C. Nitrogen	Impact on product Quality	❖ Breakdown of Air compressor ❖ Low air pressure ❖ High water content ❖ High Oil content ❖ Instrument failure ❖ Operational errors ❖ Odor ❖ Failure Particulate matter ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.

Function / Sub-function / Process Step/Unit Operation	Risk Input (Potential failure Mode)	Cause(s)
D. Water For Injection	Impact on product Quality	❖ Breakdown of WFI system ❖ High conductivity of WFI ❖ Total organic carbon Out of limit ❖ Failure of microbial test ❖ Tank vent filter failure ❖ Inadequate temperature control ❖ Instrument failure ❖ Operational errors ❖ Inadequate PLC controls ❖ Failure Particulate matter ❖ Periodic sanitization programme is not in place ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.
E. Purified Water	Impact on product /Process	❖ Breakdown of Purified water system ❖ High conductivity of Purified water ❖ Total organic carbon Out of limit ❖ Failure of microbial test ❖ Tank vent filter failure ❖ Inadequate temperature control ❖ Instrument failure ❖ Operational errors ❖ Inadequate PLC controls ❖ Failure Particulate matter ❖ Periodic sanitization programme is not in place ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.
4. Equipment A. Autoclave (Steam sterilizer)	Impact on sterilization	❖ Breakdown of autoclave. ❖ Inadequate load pattern and configuration. ❖ Inadequate qualification for Heat penetration for equipment/ Components. ❖ Monitoring of critical sterilization parameter is not in place. ❖ Load configuration changes against validated cycle. ❖ Sterilization cycle failure. ❖ Inadequate PLC control. ❖ Improper training to operator. ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.
B. Manufacturing ,filling filtration, equipment	Risk on product quality & cross contamination	❖ Improper training to operators (Human error) ❖ Improper Decontamination, cleaning & drying of an equipment's ❖ Quality monitoring programme not in place ❖ Calibration programme not in place.

Function / Sub-function / Process Step/Unit Operation	Risk Input (Potential failure Mode)	Cause(s)
C. Filter integrity tester	Impact on Product Quality	❖ Qualification of filter integrity tester is not performed ❖ Break down of equipment ❖ Change in method of testing ❖ Calibration not done ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.
D. Washing machine	Impact on Product Quality	❖ Qualification of equipment's not performed ❖ Improper training to operators ❖ Inadequate PLC controls. ❖ Qualification status is not mentioned & ensured. Unqualified equipment used or after use of qualification due date ❖ Washing of vials not done within operating range ❖ Washing of vials done at less than specified operating jet pressure. ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.
E. Depyrogenation tunnel	Risk to the depyrogenation of vials and product quality	❖ Qualification of equipment's not performed ❖ Improper training to the operators- Wrong recipe selection, change in recipe parameter ❖ Sterilization printout is not reviewed. ❖ Inadequate PLC controls. ❖ Vials Depyrogenation is not performed as per qualified recipe in BMR. ❖ Failure of HEPA Filter integrity testing ❖ Power failure ❖ Differential pressure across zone & between room is not monitored ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.
F. Filling machine	Impact on product sterility & risk to patient	❖ Qualification of equipment's not performed ❖ Inadequate cleaning and sanitization of filling machine. ❖ Alarm review is not done ❖ Power failure happened at the time of filling operation ❖ Improper training to the operators for aseptic manipulations ❖ Inadequate PLC controls ❖ Improper training to the operators & supervisors for machine operations. ❖ Improper setting of machine ❖ Routine and non-routine interventions are not simulated during batch processing increases longer exposure to the environment. ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.

Function / Sub-function / Process Step/Unit Operation	Risk Input (Potential failure Mode)	Cause(s)
G. Freeze dryer	Risk to the quality of the product	❖ Qualification of equipment not performed ❖ Alarm review is not done. ❖ Inadequate PLC controls ❖ Improper training to the operators and supervisors. ❖ Product specific recipe not followed. ❖ Defrosting process not being performed ❖ CIP process not being performed ❖ SIP process not being performed Vent filter integrity testing not performed ❖ Leak test process not being performed ❖ Breakdown for Equipment like vacuum pump and compressor. ❖ Shelf temperature for loading is not ensured ❖ FD malfunction / Human error ❖ Inadequate Supportive utilities like compressed air, clean steam, chilled water/ cooling water, purified water, water for injection, raw water. ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.
H. Capping machine	Impact on product quality	❖ Qualification of equipment's not performed ❖ Capping machine set parameter is not followed. ❖ Inadequate PLC controls ❖ Improper training to the operators ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.
I. External Vial Washing Machine	Risk for the cross contamination & human safety	❖ Improper training to the operators ❖ Inadequate PLC controls ❖ Qualification status is not mentioned & ensured. ❖ Unqualified equipment used or after use of qualification due date ❖ Washing of vials not done within operating range as per SOP. ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.
J. Vial Leak testing machine	Risk to the container closure integrity of the product	❖ Break down of leak testing machine ❖ Qualification of equipment's not performed ❖ Inadequate alarms review ❖ Leak test machine set parameter is not followed ❖ Leak testing maintenance frequency not followed ❖ Inadequate PLC controls. ❖ Improper training to the operator and supervisors for machine operations. ❖ Quality monitoring programme not in place ❖ Calibration and preventive maintenance programme not in place.

Function / Sub-function / Process Step/Unit Operation	Risk Input (Potential failure Mode)	Cause(s)
K. HVAC system	Risk on environmental control	❖ Breakdown of blower motor of Air Handling Unit (AHU unit) ❖ Pre filter cleaning frequency not defined ❖ Inadequate damper control ❖ Inadequate variable frequency drive control ❖ Stoppage of AHU ❖ Filter replacement frequency not defined ❖ Integrity testing failure of HEPA filter ❖ Air changes per hour (ACPH) ❖ Differential pressure out of limit ❖ Non-viable particle count excursion ❖ Monitoring for critical parameters are not in place ❖ Calibration and preventive maintenance programme not in place. ❖ Excursion in temperature, relative humidity and differential pressure ❖ Choking of primary and secondary filters ❖ Interruption of power supply
L. FFM /LAF	Risk on environment control	❖ Breakdown of blower motor of Laminar Air Flow (LAF)/ Fan Filter Module (FFM) unit ❖ Pre filter cleaning frequency not defined ❖ Inadequate damper control ❖ Inadequate variable frequency drive control ❖ Stoppage of LAF/FFM ❖ Filter replacement frequency not defined ❖ Integrity testing failure of HEPA filter ❖ Air Velocity failure ❖ Differential pressure out of limit ❖ Non-viable particle count excursion ❖ Monitoring for critical parameters are not in place ❖ Calibration and preventive maintenance programme not in place.
5. Materials for Aseptic Process A. Storage and usage of Sterilized materials / Equipment's	Risk of microbial contamination in aseptic area	❖ Wrapping of equipment's not done properly. ❖ Inadequate Storage of materials (silicon tubes, filters, sterile gowns, face mask, gloves etc. / equipment's after unloading into aseptic area) ❖ Hold time of sterile material not validated.
B. Storage and usage of sanitized materials / Equipment's	Risk of microbial contamination in aseptic area	❖ Items/materials which are not autoclavable, are transferred to aseptic area without following the procedure. ❖ Sanitization of material is not done. ❖ Inadequate training.
C. Sterilized Tank movement	Impact on sterilization of Tank	❖ Missed to attach 0.2 micron vent filter in inlet and outlet of tank. ❖ Storage condition of filtered bulk under LAF not followed as per the product requirement as mentioned in BMR.

Function / Sub-function / Process Step/Unit Operation	Risk Input (Potential failure Mode)	Cause(s)
D. Used Equipment transferred from aseptic area to clean area	Risk on environment and cross contamination control	❖ In appropriate transfer of used equipment's from aseptic area to clean area
E. Product contact Equipment's	Risk on microbial count and cross contamination control	❖ Improper cleaning & drying of equipment. ❖ Equipment used in multiple product ❖ Inadequate design of equipment
F. Equipment's	Impact on product quality	❖ No calibration / improper calibration in case of manufacturing tank.
G. Primary Packing Materials (Vials &Rubber stoppers)	Improper storage	❖ Non availability of facility for storage of material. ❖ Storage is not done as per recommendation.
	Vendor Approval, MOC and Specification	❖ Vendor is not qualified/evaluated, Change the MOC, Specification
	Primary packing material mix up	❖ Improper movement of the materials ❖ Improper status labeling
	Impact on product quality	❖ Inadequate analysis and release ❖ Material may not be of right specification, Product failure
H. Rubber stoppers	Impact on rubber stopper sterilization and drying	❖ Inadequate verification for intactness of rubber stoppers bag. ❖ Inadequate wrapping of rubber stopper ❖ Rubber stopper sterilization method not followed ❖ Load configuration is not followed as per qualified load as mentioned in the SOP. ❖ Inadequate unloading and storage of sterilized bag
I. Depyrogenation of vials	Impact on Sterilization and depyrogenation of vials	❖ Sterilization and depyrogenation of vials not performed as per defined recipe in BMR
J. Sterilizing Grade Filters	Inadequate filters	❖ Filter taken wrong which was not mentioned in BMR for filtration process. ❖ Selection of wrong filter which leads to incompatibility resulting in increased leachable from filter, adsorption of API or preservatives. ❖ Filter integrity not checked for hydrophobic/ hydrophilic filter, may be product filter, gas filter or air vent filter.

Function / Sub-function / Process Step/Unit Operation	Risk Input (Potential failure Mode)	Cause(s)
K. Silicone tube / FEP tube	Inadequate Silicone tube / FEP tube	❖ Improper cleaning and dryness of tube. ❖ Heat treatment of tubing is not carried out for three times. ❖ Tubes are reused for second batch & not discarded after first batch processing. ❖ Improper sterilization leads to impact on sterility assurance. ❖ Wrong selection of ID x OD
L. Dispensing Raw material (API & Excipients)	Impact on Material	❖ Raw material quantity not dispensed as per master formula. ❖ Use of non-calibrated balance, calculation error. ❖ API is not dispensed in Specified container (light protective container). ❖ Dispensing not done as per mention in MF and BMR condition. ❖ Selection of wrong material / under test material / rejected material. ❖ Dispensing without verification of raw material. ❖ Vendor not qualified / unproved ❖ Improper movement of the materials lead cross contamination
6. Manufacturing process A. Bulk manufacturing	Impact on product quality	❖ Over exposure of bulk solution in closed SS tank against specified in MF and BMR. ❖ Bulk hold time / SS compatibility. ❖ Human error/instrument error during sampling ❖ Volume make up is not done properly ❖ Product Specific precautions not followed
7. Sterilization process A. Equipment sterilization	Impact on product quality	❖ Equipment's used after 48 hours of sterilization ❖ Equipment's cleaning and sterilization not performed. ❖ Cleaning process is not followed as per SOP. ❖ Sterilization parameters are not achieved as per the standard cycle. ❖ Load configuration is not followed as per qualified load as mentioned in the SOP
B. Garments for Aseptic area	Impact on Environment	❖ Inadequate cleaning and sterilization of dresses. ❖ Inadequate integrity/ intactness of dresses.
8. Filtration process (Bulk Filtration) A. Assembling process – Filtration stage	Impact on product quality	❖ Inadequate aseptic interventions like unwrapping of sterilized equipment's, aseptic connection, transfer of tube & hand sanitization. ❖ Wrong filter selection / use. ❖ Total Filtration time exceeds than validated time. ❖ Rate of filtration, pore size of filters may change. ❖ Filtration assembling load used after exceed hold time

Function / Sub-function / Process Step/Unit Operation	Risk Input (Potential failure Mode)	Cause(s)
B. Pre integrity of Filter	Impact on product quality	❖ Failure of pre integrity test due to wrong assembly, ❖ Usage of damage filter, improper wetting & wrong technique followed during testing ❖ Temperature of the fluid not monitored at the time of filter integrity test
C. Filtration Process Parameter	Impact on product quality	❖ Filtration parameters not match & filtration procedure is not followed as per BMR. ❖ Peristaltic pump not operate as per defined speed in BMR. ❖ Total filtration time & rate of filtration is more than the specified limit. ❖ Pre-post nitrogen purging is not carried out with sterile hydrophobic filter. ❖ Integrity testing of hydrophobic filter for gas purging is not being performed and no. of cycles is not being monitored. ❖ Bio burden testing not performed
D. Post filtration integrity of filter	Impact on product quality	❖ Failure of post integrity test due to wrong assembly, ❖ Usage of damage filter, improper wetting & wrong technique followed during testing
E. DFA filter & Vent filter attached with filling tank after filtration	Impact on product quality	❖ Integrity of the air vent filter/ DFA filter is failed/not performed / not monitored for no. of cycles. ❖ Aseptic interventions like attachment of DFA filter with the tank of vent filter, aseptic connection, hand sanitization not carried out as per SOP
F. Movement of tank from filtration room to filling room	Impact on Environment	❖ Missed to attach 0.2 micron vent filter in inlet and outlet of tank
G. Storage	Impact on product quality	❖ Storage condition of filtered bulk under LAF not followed as per the product requirement as mentioned in BMR
9. Filling Process	Impact on product quality and sterility assurance	❖ Operators & supervisors of Aseptic area have not undergone training related to gowning, de-gowning. ❖ Inadequate environmental monitoring during filling process. ❖ Inadequate sanitization process. ❖ Inadequate sterilization of filling equipment. ❖ Inadequate change parts selection. ❖ Inadequate assembling of equipment parts. ❖ Inadequate intervention handling. ❖ Breakdown and machine stoppage during the filling process. ❖ Inadequate barrier control during filling process. ❖ Inadequate quality monitoring program. ❖ Inadequate process for material transfer. ❖ Limit for time, personnel and filling speed not defined. ❖ Media fill simulation frequency not defined/followed.

Function / Sub-function / Process Step/Unit Operation	Risk Input (Potential failure Mode)	Cause(s)
10. Lyophilization Process	Risk to the quality of the product.	❖ Qualification of equipment not performed ❖ Improper training to the operators and supervisors. ❖ Defrosting process not being performed ❖ CIP process not being performed ❖ SIP process not being performed Vent filter integrity testing not performed ❖ Leak test process not being performed ❖ Product specific recipe not followed. ❖ Quality monitoring programme not in place
11. Capping Process	Impact on product Quality	❖ Vials are cracked or broken due to overpressure during capping ❖ Equipment and line set up procedure is not followed ❖ Improper training to the operators. ❖ Capping machine not working properly ❖ Container / closure integrity is not performed for respective filling configuration.
12. External Vial washing Process	Impact on product Quality	❖ Vials are not cleaned properly due to lower pressure of washing media ❖ Equipment and line set up procedure is not followed ❖ Improper training to the operators
13. Leak test of vials: Leak testing	Impact on sterility assurance of the product	❖ Improper training to the operators ❖ Wrong recipe selection for leak testing
14. Visual inspection Procedure	Impact on product Quality	❖ Improper training &Qualification of visual inspectors. ❖ Lux level (Exposure to light) and black & white film of visual inspection booth is not checked as per schedule. ❖ Eye sight checking frequency is not defined. ❖ Visual inspector eye rest time frequency and practices is not followed. ❖ Procedure not followed as per standards. ❖ Minimum time criteria for visual inspection not defined. ❖ AQL testing plan not in place ❖ Classification and training of defects is not carried out.
15. Smoke study – Air flow pattern procedure	Impact on environment and sterility assurance of the product	❖ Improper training to the operators ❖ Inadequate procedure for smoke study. ❖ Inadequate execution of smoke study
16. Media fill simulation process	Impact on sterility assurance of the product	❖ Inadequate media fill procedure ❖ Inadequate frequency of media fill ❖ Inadequate selection of worst case ❖ Interventions are not simulated ❖ Inadequate operator training and participation ❖ Inadequate duration of media fill ❖ Inadequate rejection criteria ❖ Inadequate accountability ❖ Inadequate visual inspection ❖ Inadequate incubation ❖ Inadequate microbial testing

Function / Sub-function / Process Step/Unit Operation	Risk Input (Potential failure Mode)	Cause(s)
17. Batch disposition Procedure	Risk on product quality, sterility assurance and patient safety.	❖ Procedure not followed as per standards ❖ Inappropriate review of Batch record. ❖ Inadequate training for batch record review.

Appendix II
Interventions Trending and Evaluation

The firm has to define the procedure and protocol for evaluation and trending of the interventions (Routine and Non-routine) for identification of the interventions, to determine the frequency and duration of the interventions for its further requirement of the simulation in subsequent media fills (Process simulations).

A. Study Plan and procedure

- The assessment must include intervention (routine and non-routine interventions) details like type, frequency and duration of intervention for each commercial batch produced on a particular line for concerned process and container size since last successful media fill run.

- Collect the number of batches that are commercial/exhibit/trial batches manufactured since last successful media fill.

- List down the interventions (routine and non-routine interventions) frequency and duration at least 25 preceding commercial batches or six month of batches whichever is higher as per provided in below format.

- If the batches are less than 25 batches, then collect the data from available similar line and all executed batches since last media fill.

- If the batches are less than 25 batches and similar line is not available then perform brain storming with QA, Micro and engineering team. And based on the brainstorming, list out the identified interventions provided table format.

- If the batches are more than 25 then list down the interventions of all executed batches since last media fill.

- Trend should be evaluated against the media fill metric of the interventions, to check the max validated duration and maximum frequency of the respective interventions, validated interventions and operators involved for handling of the interventions.

- If any new intervention observed, which is not validated and simulated in the media fill and not in the media fill metric of the interventions, then initiate the deviation and performed the impact assessment for appropriate corrective action, and include it in the media fill protocol for simulation in subsequent media fill.

- If the Intervention frequency and duration exceeded from previous media fill batches/ Media fill metric of the interventions, then investigation to be performed for impacted batch in which discrepancy observed and media fill Protocol revision is required before execution of next media fill.

- If the Intervention frequency and duration does not exceed from previous media fill, then media fill Protocol revision is not required.

- Prepare the trend for interventions frequency and duration of available data, if any abnormal trend observed then same is to be investigated as per SOP “Deviation process”.

- If any additional evaluation required maximum batch size to be considered for the interventions details and duration.

- After evaluation and trending, it should be documented and approved by quality units and necessary corrective actions to be concluded.

- If any of the intervention is atypical and significantly excessive than routine / other batches then the same need not necessarily required for inclusion in media fill to validate increased frequency of concerned interventions.

- The limit of number of intervention (Frequency) in media fill protocol and BMR will remain unchanged.

- In case there is a requirement to increase frequency of intervention (number of interventions) for increase in batch size, change in pack size of component e.g. rubber stopper, bottle, dropper, cap etc. and/or there is any change in process then the rationale for the same to be prepared and the increase in intervention to be approved by site Quality Head.

Details	Observation
Block No./ Facility Name	
Container and closure size	
Process (e.g. lyophilized/liquid/ suspension/PFS etc.)	
Room Number / Line No.	
Equipment ID :	
Duration considered (From (DD/MM/YY) to (DD/MM/YY)	
Total number of batches included	
At least 25 preceding commercial batches or six month of batches whichever is higher (Include the trial batches /exhibit data during evaluation of intervention trends and duration ,Only in case of non-availability of 25 nos. of commercial batches data)	

If any other similar line data taken for interventions frequency and duration then mention the below details	
Line details	Observation
Block No	
Container and closure size	
Process (e.g. lyophilized/liquid/ suspension/PFS etc.)	
Line Number	
Rationale for similar line selection	

Batch No.	Type of Interventions (Routine/ Non-routine)	Intervention Name	Frequency & Duration of interventions			Handing with Open/ Close door	Door Number/ Glove port number
			Max. no. of interventi on	Max Time (Min)	Min. Time (Min)		

MEDIA FILL METRIC OF THE INTERVENTIONS
[Based on the media fill batches (Process simulation)]

Type of Interventions (Routine/ Non-routine)	Intervention Name	Operators (Who) involved in simulation of this intervention	Frequency & Duration of interventions			Door Number	Glove port number
			Frequency	Duration	Open/ Close Door		

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