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Review Article

DESIGNING AND QUALITY ASPECTS OF ASEPTIC PROCESS SIMULATION

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ABSTRACT

Aseptic process simulation is a crucial validation technique carried out before a new product or aseptic process is introduced in the facility and also to prove on regular intervals that the existing manufacturing operations are carried out in a state of aseptic conditions. Aseptic process simulation involves conducting aseptic production using a sterile growth medium instead of actual drug solution and excipients. The processes involved in aseptic validation include the identification of process mechanisms, variables and control methods and that also include product, component, and sterilization of equipment, sanitary facilities, environmental checks and staff training on gowning procedure. This review addresses the nature of the study involved in aseptic process simulation, speed and number of runs, runtime, the atmospheric conditions, line speed, the media used, incubating and analyzing media-filled units, data interpretation, worst-case parameters, interventions, case study on interventions and the regulatory aspects concerned with the simulation.

Keywords: Aseptic Process Simulation, Validation, Media, Interventions, Gowning procedure

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INTRODUCTION

An Aseptic process simulation (APS) is mimicking the steps starting from the dispensing of the raw material to the point products are sealed with sterile growth media, it starts with the formulation and ends with container closure; thus an APS is simply a process simulation of the exact formulation process of the drug product. This process simulation employing growth media instead of the actual drug product is also known as media fill [1]. Broth fills, media fills, simulation trails, are the synonyms for the activity undertaken as a result of the validation and repeated testing of the latest aseptic technology [2].

Media fill studies should be carried out under the worst instance conditions, including maximal processing and filling time and must include simulation of all aseptic manufacturing processes.

There are two different situations at which the aseptic processing is applied:

Preparation and filing of solutions aseptically,

Aseptically transferring, handling and packaging of solid products that are not sterilized terminally in their final containers.

The simulation media filling process is state-of-the-art to verify the aseptic production process in compliance with the USFDA and EU Guidelines. The filled container and closure are finally checked for microbial contamination by incubating under defined parameters. This is to confirm that the product is manufactured under a very less contamination rate. All the regulatory authorities across the globe state that the manufacturing procedures in the industry can be started only after validating aseptic process simulation [3].

Steps involved in media fill validation process

Selecting suitable growth media [4]

Demonstration of nutrient growth promotion properties

Environmental monitoring

Formulation of bulk media solution [5]

Microbial performance and bio-burden examination

Sterilization of the media using suitable sterilization techniques

Filling of media into containers

Filled vials must be incubated and evaluated

Microbial requirements on validation of medial fill

The liquid state nutrient growth medium supports the growth of microorganisms, which is used after the sterilization process and filled to mimic normal production processes that follow the steps like

Compounding

Sterile filtration

In-process control checks

Sterilization of the manufacturing process

Sterilization of materials: garments, primary containers, filling equipment by using various sterilization techniques like autoclaving, dry heat or gamma radiation [6].

Cleaning, sterilization process and filling [7].

The control checks performed to maintain the hygienic state of the manufacturing process are:

HVAC validation

Qualification of personnel

Gas supply systems

Water handling systems

Equipment washing and sterilization [2]

Purpose of aseptic process simulation

The food and drug administration states that an APS must validate the aseptic method by using the growth medium, which is both handled and exposed in an identical way to personnel, machinery, surfaces and environmental conditions. The purpose of APS by the parenteral drug association is to certify and qualify aseptic processing personnel [8].

The purpose of the simulation is to provide the probability of microbiological contamination caused in a specific aseptic process. The ampules or vials are filled with medium. The packed containers are incubated and the numbers of contaminated units are rated against the number of uncontaminated units, thus providing an index of the probability of contamination (the contaminated proportion) resulting from the aseptic phase [3].

The major microbial contaminations in aseptic processing are personnel contaminants, human error, non-routine activity, aseptic assembly, mechanical failure, improper sanitization, material

transfers, surface contamination, airborne contamination, Failure of 0.2µ filter, failure of HEPA filters, improper sterilization [4, 8].

Study analysis during aseptic process simulation

Pre aseptic process simulation activity

Before starting the APS activity, the Pre aseptic process checks must be conducted to avoid the microbial growth, entry of particulate into an aseptic area and also conducting of swab sampling before the equipment starts with its manufacturing process.

Microbial monitoring

In and around high operator activity areas, microbial control should be carried out. It is not uncommon to see plate settlements and positions of air samples far away from those regions. A standard check is the location of settle plates on the rear of the filling machine where there is little or no operator involved.

Particulate monitoring

Routine particle tracking is useful for the rapid identification of major air cleanliness deviations from eligible processing requirements (e. g. destination of clean areas) [5, 9]. The particulate monitoring involves the monitoring of the non-viable and viable particles which come under environmental monitoring. If the movement of personnel is more in the aseptic area, particulate matter increases.

Swab sampling

The cleanroom areas must be monitored to regulate the microbiological cleanliness of Grades A to D during manufacturing. Monitoring should be periodic, where aseptic operations are performed using processes like settle plates, surface, and volumetric air sampling [6, 10]. The swab sampling is done before the aseptic processing activity starts. Sampling is mainly taken from the equipment that is used in the manufacturing process. Generally, the sampling procedure is done by both in-process quality assurance personnel and the microbiologist. Microbiologist checks the growth of micro-organism then assures with the next process of manufacturing.

During the aseptic process simulation activity

During the manufacturing process, additional precautions need to be taken to mimic the exact interventions that might the aseptic condition of the manufacturing facility. Some of the factors include,

Gowning procedure

Gowing is important in aseptic processes to prevent contamination [7, 11]. The training of the employees for gowning practices should be assisted by an observer who visits the aseptic cleanroom to confirm that correct procedures are practiced [8, 12]. The analyst must wear a clean laboratory suite, sterile sleeves and sterile gloves when doing the examination. Disinfected gloves must be used for the opening and handling of the sample units [9, 13]. The gowning practice in the aseptic manufacturing area must be practiced as per the following fig. 1 given below

Fig. 1: Gowing procedure [10, 14]

Disinfection

It is important to determine the suitability and efficacy of the disinfecting agents and its procedure. The efficacy of the disinfectants and procedures should be evaluated in terms of their ability to ensure that possible contaminants are extracted from surfaces sufficiently [15]. To avoid contamination, disinfectants must be sterile, properly maintained in suitable containers and not used for longer than the duration specified in the written protocol [5, 9].

Personnel movement

While concerns of cleanroom apparel are usually reduced in an isolator process, it is not possible to ignore the contamination risk posed by manual factors. Isolation procedures usually require occasional or even regular use of one or more gloves to manipulate aseptically and manage the material transfer to and from the isolator. To decrease the contamination of the product, personnel movements must be avoided in the aseptic manufacturing area. Improper personnel movement can lead to the risk of contamination.

Post aseptic process simulation activity

After completing the APS activity, the post aseptic process checks must be conducted to evaluate the possibility of microbial growth, entry of particulates into an aseptic area and also conducting of swab sampling after the manufacturing process. Performing this post-simulation activity gives a better picture of possible contaminants at the time of manufacturing.

Factors to be considered during aseptic process simulation

Water system qualification

By using purified water, water for injection must be produced.

They must be stored and distributed properly to prevent microbial growth by maintaining constant circulation at a temperature above 70 °C.

Different methods like distillation, post reverse osmosis, ultra-filtration and Nanofiltration are the techniques used for the preparation of water for injection.

By considering seasonal variation, the water system must be approved to control relevant levels of microbiological growth.

Turbulent water flow must be maintained through the pipes to prevent microbial adhesion [16].

Pure steam qualification

For pure steam production, sterilized water with lower levels of endotoxins must be used.

Steam used to sterilize shall be of appropriate portion and must not contain contaminants that are capable of contaminating the equipment or substance at a point.

Compressed air system qualification

The primary product or container surface in direct contact with compressed gasses must have related components, particulate and microbiological purity without oil with adequate requirements for dew points and acceptable pharmacopeia. At the point of use compressed gases should be filtered via sterilization filter with a determined pore size of 0.22 µm.

Elements involved in aseptic process simulation

There are various aspects to be considered to successfully conduct an APS validation study, the parameters to be selected can be based on the Risk assessment carried out in determining the factors affecting the aseptic filling [17]. Fig. 2 represents a few of the factors which might be considered in the process of validation.

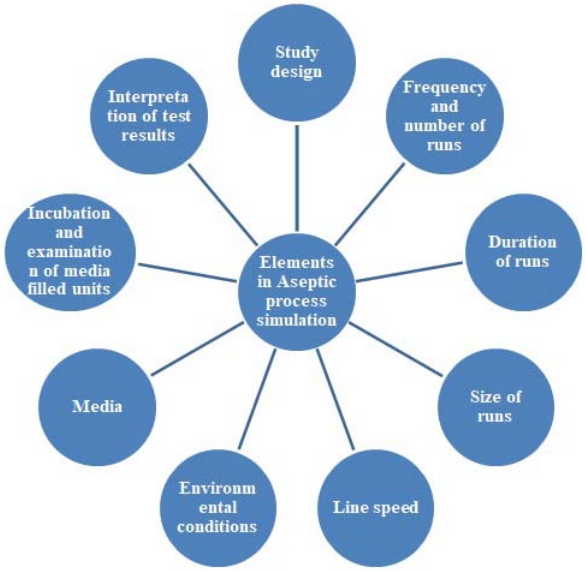

Fig. 2: Elements in APS [9]

Aseptic process simulation study design

The media fill program includes risk factors for the contamination that happens on a manufacturing line, precisely measures the state of method control. Media fill studies must carefully pretend aseptic developed processes including, worst-case actions, circumstances that offer a task to aseptic operations. Recommendations and issues that are provided by the FDA are [18].

Issues relating to the longest appropriate processing line run will lead to a risk of contamination. (Tiredness of operator can be given as an example)

Aseptic assembly of equipment's

Number of activities and their associated personnel

Weight checks

Speediness and structure

Container closure system

Aseptic sample gathering

Frequency of media fills

In the process simulation procedure, the frequency of media fills should be fixed based on risk assessment.

Initial process simulation

For new facilities, equipment, filling line and container design, initial performance testing is performed. As per Japanese Pharmacopoeia, Media fill testing shall be conducted with appropriate liquefied products that have been filled into units that correctly represent a real one-manufacture filling line with at least three repeat runs on different days. For bulk product, the check should be carried out using the bulk product quantity of one production unit [19, 20].

Repeated process simulation

Periodically conduct a media filling requalification at each work change for the filling line. Aseptic processing workers must be qualified to carry out aseptic processing and participate in media filling.

If filling lines are not used for more than six months, then perform enough medium fills in the same way as the initial output certification before resuming the filling lines.

In the case of facilities and equipment improvements (interchangeable parts do not need requalification), adjustments in personnel involved in essential aseptic manufacturing (e. g. new crews) discrepancies in environmental test results, or a product sterility check indicating contaminated goods, the correct number of media fill runs is performed in the same manner as in the original performance testing before the adjustment [19].

Duration of runs

In media fill design, the length of aseptic handling operation is the main consideration. While the utmost realistic simulation model should be the maximum lot size and length since the actual manufacturing process is more closely simulated, other acceptable models may be reasonable [21].

The time needed to implement factors and initiatives shall be measu red according to the duration of the media fill, as real aseptic processing activity duration must be taken into account.

Commonly occurring interventions must be simulated routinely, while those rarely occurring may be simulated periodically.

The FDA suggests opening unsealed containers to partial chamber evaluation in a manner that would mirror the protocol for lyophilization operations.

Vials must not be frozen, and precautionary measures must be reserved to certify that the medium is leftover in an aerobic manner to escape micro-organism growth being possibly inhibited [9, 22].

Size of runs

During the simulation phase, the number of units performed must depend on the risk of contamination of the specified process and be adequate to simulate activities representative of the production process.

An appropriate initial point for run size is in between the series from 5,000 to 10,000 units. The number of filled units must be at least equivalent to the regular lot size produced in the manufacturing line for operations with an output of sizes below 5000 [9].

When the potential for contamination is greater based on process design, several units with full batch size must be used. An isolator cycle may have a smaller risk of contamination as a result of the lack of direct interference by humans and may calculate with fewer units as a percentage of total operations.

Because several batches are created over various shifts or outputs and an exceptionally high number of units, media filling is especially important. In designing the simulation to accurately cover the

circumstances and any potential risks associated with the larger operation, these considerations are carefully considered [23-25].

Line speed

The media fill system will discuss appropriately the line speed range used during development, but the media fill run will determine a single line speed and validate the chosen speed [9]. For instance, the use of high line speed is frequently more acceptable when assessing the production method influenced by repetitive interventions or a considerable degree of physical manipulation. Using slow line speed is typically suitable for testing production processes with extended sterile drug product exposure and containers/closures [18, 23].

Environmental conditions

Monitoring of non-viable particles

Monitoring of the chosen location must be observed to ensure the worst-case conditions.

The room monitoring is done where the activity of the operator is more and the particulate counts in the location must be performed.

The checking of the environmental counts must be performed adjacent to the filling zone and to investigate the operator activity within these areas [21].

The air from the HEPA filter is monitored with sampling probes.

The sampling device must not compromise the airflow in the critical zone.

The worst-case conditions are identified and confirmed by the validation process.

By process simulation tests these are reconfirmed [18, 26].

Media

Soybean casein digest medium (SCDM), which promotes gram-positive and gram-negative growth of bacteria, yeast and mold must be used [9].

In special circumstances, consideration should be given to the use of anaerobic growth media (e. g. fluid thioglycollate medium).

Environmental inspection and sterility checking can be replaced or applied to the growth-promoting factor.

Growth-promoting units must be inoculated with a<100 CFU challenge. Nevertheless, if the growth promotion test fails, it is important to examine the root of any contamination detected during the simulation, immediately repeat the media [13].

The manufacturing process must be simulated precisely using media and circumstances that minimize microbiological contamination.

The individual unit has to be filled with an acceptable quantity and type of microbial growth medium to detect microbial growth visually on the inner surfaces of the container (when the unit is flipped or thoroughly whirled) [14].

The below fig. 3 indicates the initial stage of the test regarding the SCDM with no growth of microorganisms and fig. 4 shows the growth of microorganisms after completion of inoculation and the incubation. A growth indicates that the media is supporting the growth of organisms and the media can be chosen for the fill studies.

Fig. 3: Soybean casein digest medium before inoculation [27]

Fig. 4: Growth of microorganism in soybean casein digest medium after inoculation and incubation [28]

Incubation and examination of filled units

Incubate all the media fill units at 20 to 25 °C for 7 d. The incubation rage must be within 22.5±2.5 °C [29].

After the completion of 7 d at the 20 to 25 °C, for the next 7 d, containers are placed in an inverted position and incubate them at 30 to 35°C. The temperature incubation range must be maintained within 32.5±2.5 °C [30].

After day 3, day 7, day 10 and day 14, the qualified microbiologist examines each Filled media unit.

During the observation, all the suspected units are sent for investigation to QC microbiologist.

All suspicious units recognized during the investigation must be carried to the instant consideration of the QC microbiologist.

QC microbiologist may recommend exchanging transparent containers for amber or other opaque containers to detect microbial growth easily [9].

Units that are not found to have faults linked to integrity must be incubated and units lacking integrity are rejected.

Interpretation of data

Table 1: Interpretation of data

Range	Acceptance criteria	Reference
If the range of filling is less than 5000 units	The units determined must be of no contamination.	[5]
When filling range is in between 5000-10,000 units	•Including the consideration of repeat, media fill only one suspected unit should be found in an investigation. •Two contaminated units are deemed cause for revalidation following an investigation.	[15]
If the filling range is more than 10,000 units	•In an investigation only one contaminated unit must be found. •Cause for revalidation involves the consideration of two contaminated units.	[25]

The below image gives the information regarding the clear vial and the contaminated vial

Fig. 5: Images of contaminated and uncontaminated vials [32]

Worst-case parameters or conditions

Worst-case scenarios in the sense of aseptic processes do not essentially mean the worst-case likely. To get technical benefits after the analysis, the worst-case scenarios must be used in the simulation readings of the process [21].

The simulation design will suit the routine aseptic manufacturing process as closely as possible and should include all relevant essential development steps. Reasonable combinations of container size and aperture, as well as transmission line speed, should be used. The simulation test for the process will represent a worst-case scenario and include all manipulations and procedures that are likely to be encountered during a changeover.

Worst-case parameters are defined for the machines which are used as shared equipment's which involves [26]

Longest fill duration

Wide container opening

The longest exposure time of open container

Case study on considering the worst-case in media fills

Do the minimum and the maximum number of operators in the cleanroom considered as the worst-case?

Yes, more number of operators is a large source of microbial contamination, but a limited number can increase operator activity and reduces focus. Each can be seen as the worst-case.

What can be the worst-case between the larger containers and smaller containers?

Both might be considered as worst-cases because the chance of contamination is more into the larger containers having wider openings and throughout the transportation and filling process, smaller containers are not stable.

Which can be chosen as the worst-case slowest or fast-fill speed?

In this case, the slower filling represents more risk contamination before sealing, the faster filling can represent the more mechanical movement, potential airflow disruption, and the transport of containers is unsafe.

From these parameters, a single set of worst-cases can't be chosen, for this reason in the process simulation analysis, all possible worst-case configurations must be included [18].

Intervention monitoring

The interventions are classified as:

Interventions caused by humans in aseptic processing

Routine Interventions

Non-routine interventions [33]

Interventions caused by humans in aseptic processing

The interventions caused by humans have more potential impact on aseptic processing when compared with other factors contamination.

Room design, sterilization processes, environmental sanitation, heating and ventilation are the sources that provide less contamination.

The organisms present on humans are in millions and these microorganisms are spread continuously to their gowning materials and their surroundings because of improper gowning

Improper personnel Hygiene

Eating, drinking, smoking on-premises, etc.

Routine interventions

These are the activities that occur in every batch and can also be named as typical routine interventions

Equipment must be aseptically assembled

Start-up or launch of the part

Weight or volume change of the initial fill

Periodic component replenishment

Periodic refilling of the components

Periodic filling and measuring weight or volume

Correction of volume or fill weight

Monitoring of environmental conditions

Operator breaks and meals

The change in shift of the operator

A Sampling of the product

The integrity of the filter is tested

Replacing product container

Adjustment in a component [8]

Non-routine interventions

These interventions are not part of every batch, but they are predominantly corrective.

Avoid clumping or misfeed of the stopper

Collapsed, wrecked, or blocked containers

Remove faulty seals on containers

Leakage of the product must be avoided

Product Filter change

Sensor adjustments or replacement

Replace the filling indicator

Additional fill-pump must be used alternatively

Schedule changes

Changes to Conveyor or guide rail [31, 33]

Case studies on interventions

Case study 1

Interventions produced by personnel

Background

More than half of the units that are run by media fill were found to be microbiologically contaminated. Minor changes were made to the process and three media fills were carried out again. A high level of contamination was yielded during the second media fill run. Isolates in both failures were common skin borne microbes.

Issues found by cGMP

cGMP observes that there is an improper gowning practice followed by the personnel in the aseptic area. The problem that is found in the contamination was a design flaw in the gown.

Action

The firm corrected the deficiencies recognized in the gowning practice.

Case study 2

Assuring container-closure integrity throughout manufacturing

Background

Parenteral drug products have been found infected with Enterobacter cloacae and analyses of sealed vials have improved *Xanthomonas maltophilia* microorganism.

Issues found by cGMP

They found that there is a container closure integrity problem. The sealed glass vials that had been passed through secondary packaging are dropped from the bulk pallets by the operator. In the cleaning process of the spillage product, the personnel washed the unbroken vials with portable sink water.

Root cause

The problem that is identified in this case is packaging and labelling. Root cause observed was that improper handling of the sealed glass vials and harsh treatment of these may result in small cracks on vials. The microorganisms enter the product when the vials are washed with potable water. The water samples are collected by FDA from the firm and the same organism *E. cloacae* was identified [34].

CONCLUSION

The simulation process should stimulate the production process, where the aseptic activities are performed, from the establishment of vial assemblies through to the transfer of the bulk drug from the sterilizing filter to the finished containers that are ready for release. Regulatory authorities very closely monitor the media fill qualification studies as they form the base for future commercially manufactured products. Any failure to qualify media fills is considered as a serious issue and costs a lot to the manufactures as the production needs to be halted until the firm clears the media fill study. So at most, care has to be taken while planning, designing and executing media fill studies, which can be achieved through experience and proper execution.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Eurofins Scientific. Media Fill Studies. Available from: https://www.eurofins.com.au/biopharma-services/testing-solutions/sterile-products-testing/media-fill-studies/ [Last accessed 22 Dec 2019]
2. Kevin L Williams. Microbial contamination control in parenteral manufacturing. Taylor and Francis e-Library; 2005. p. 140.
3. Dgra de. Basic requirements for aseptic manufacturing of sterile medicinal products. Available from: https://dgra.de/media/pdf/studium/masterthesis/master_greger_g.pdf [Last accessed on 01 Feb 2020]
4. Devaraj B, Kali A, Charles MVP, Srinivasaiah Seethe K. Modified biphasic media for blood culture. Asian J Pharm Clin Res 2016;9:3
5. Diniatik, Sundhani E, Taufiq Nugroho M, Nur Hikmah E, Wahyuningrum R, Suparman. Antifungal and antibacterial activities of juice and ethanolic extracts of garcinia mangostana l. Leaves. Asian J Pharm Clin Res 2017;12:7
6. Silva B, TV Araujo, P Vasconcelos, H De Paula, N Nogueira, C Lira, *et al*. Gamma radiation as a method for sterilization of all-in-one admixtures bags for clinical use: a study of stability. Int J Pharm Pharm Sci 2015;7:129-35
7. Rubashvili I, Karukhnishvili N, Loria K, Dvali N. Cleaning validation for estimation of active ingredients' residues of vicazid uncoated tablets (pyrantel 100 mg/mebendazole 150 mg) on surfaces of pharmaceutical manufacturing equipment using swab sampling and HPLC method. Int J Pharm Pharm Sci 2015;7:6
8. Deshmukh A. Aseptic process simulation: an assessment of aseptic processing capability. World J Pharm Res 2018;7:609-26.
9. Fda.gov. Guidance for industry sterile drug products produced by aseptic processing current good manufacturing practice; 2004. Available from: https://www.fda.gov/media/71026/download [Last accessed 29 Nov 2019]
10. Who.int. WHO good manufacturing practices for sterile pharmaceutical products; 2011. Available from: https://www.who.int/medicines/areas/quality_safety/quality_assurance/GMPSterilePharmaceuticalProductsTRS961Annex6.pdf [Last accessed on 17 Jan 2020]
11. Cleanroomtechnology.com. Managing aseptic gowning within classified environments. Available from: https://www.cleanroomtechnology.com/technical/article_page/Managing_aseptic_gowning_within_classified_environments/107339. [Last accessed on 21 Jan 2020]
12. Pharmaceuticalonline.com. How to Establish an Aseptic Gowning Qualification Program. Available from: https://www.pharmaceuticalonline.com/doc/how-to-establish-an-aseptic-gowning-qualification-program-0001 [Last accessed on 14 Feb 2020]
13. Fda.gov. Pharmaceutical microbiology manual; 2014. Available from: https://www.fda.gov/files/about%20fda/published/Pharmaceutical-microbiology-manual.pdf [Last accessed on 09 Feb 2020].
14. Dcvmn.org. Aseptic processing practices and process validation of aseptic operators. Available from: https://www.dcvmn.org/IMG/pdf/cbe_ 107_aseptic_processing_v1_dcvmn_beijing.pdf [Last accessed on 19 Dec 2019]
15. Kanchan C, Sangeeta D, Balasaheb G. A comparative survey of knowledge of antiseptic and disinfectant use and effect of intervention on medical and surgical staff nurses in a tertiary care hospital. Asian J Pharm Clin Res 2018;11:1
16. Picscheme.org. Pic/s guide to good practices for the preparation of medicinal products in healthcare establishments; 2014. Available from: https://www.picscheme.org/layout/document.php [Last accessed on 12 Dec 2019]
17. Mahdiani H, Doroud D, Shahali M, Pouyan N, Brufar F, Rahimi A, *et al.* A practical risk-based approach to assess vial's dimensions deviations effect on the aseptic filling processing, according to ich q9 guideline. Int J Pharm Pharm Sci 2014;6:9
18. Igor Gorsky, Harold Baseman. Principles of parenteral solution validation. 1st ed. United States; 2014.
19. Jpdb.nihs.go.jp. Japanese Pharmacopoeia. 14 ed.; 2001. Available from: http://jpdb.nihs.go.jp/jp14e/14data/Medial_Fill_Test.pdf [Last accessed on 24 Nov 2019]
20. Pmda.go.jp. Guidance on the manufacture of sterile pharmaceutical products by aseptic processing; 2006. Available from: https://www.pmda.go.jp/files/000153543.pdf [Last accessed 06 Dec 2019]
21. Picscheme.org. Validation of aseptic processes; 2011. Available from: https://pi007_6recommendationonasepticprocesses.pdf [Last Accessed on 16 Dec 2019]
22. Express I. Express pharma. Vol. 11; 2016. Available from: https://issuu.com/indianexpressgroup/docs/ep-20160131pages [Last Accessed on 16 Dec 2019]
23. Gmpua.com. Good manufacturing practice. Available from: https://gmpua.com/index_en.php [Last accessed on 25 Feb 2020].

24. Pdfs.semanticscholar.org. Available from: https://pdfs.semanticscholar.org/f9e4/4891aa9314a30fcf05b1c19e1ca7c55054c0.pdf [Last accessed on 05 Mar 2020]
25. Kolhe P, Shah M, Rathore N, York S. Sterile product development-formulation, process, quality and regulatory considerations | parag kolhe | springer. Springer. com.bfsioa.org. Validation of aseptic fill process. Available from: https://www.bfsioa.org/media/12ad469f-23d9-4511-a5ab-a8adec9b8071/7mVnzw/2018%20Kunming/Validation%20of%20aseptic%20fill%20process.pdf [Last accessed 08 Feb 2020]
26. Bfsioa.org. Validation of aseptic fill process. Available from: https://www.bfsioa.org/media/12ad469f-23d9-4511-a5ab-a8adec9b8071/7mVnzw/2018%20Kunming/Validation%20of%20aseptic%20fill%20process.pdf [Last accessed on 08 Feb 2020]
27. Bio-equip.cn. Soyabean casein digest agar with lecithin and polysorbate 80, 90 mm (ASCDA002), specification, price, image-Bio-Equip in China. Available from: https://bio-equip.cn/enshow1equip.aspequipid=71194 [Last accessed on 13 Feb 2020]
28. Pharmasopcorner.net. Available from: https://www.pharmasopcorner.net/2019/09/soyabean-casein-digest-agar.html [Last accessed on 04 Feb 2020]
29. Choudhary A. Media fill validation-SVP. Pharmaguideline.com. Available from: https://www.pharmaguideline.com/2011/04/media-fill-validation-svp.html [Last accessed on 18 Jan 2020]
30. Cleanroomtechnology.com. Media fill simulations; 2014. Available from: https://www.cleanroomtechnology.com/news/article_page/Media_fill_simulations/103050 [Last accessed on 02 Mar 2020]
31. Sandle T. Designing aseptic process simulations: the time and container number conundrum. Ivtnetwork.com; 2016.
32. Biotechnologysolutions.com. Phenol red dextrose (PRD)–BTS–biotechnology Solutions. Available from: https://biotechnologysolutions.com/phenol-red-dextrose-prd/ [Last accessed on 03 Mar 2020]
33. J Agalloco. Managing aseptic interventions. J Pharm Technol 2005. p. 56-66.
34. Richard L. Friedman. Aseptic processing contamination case studies and the pharmaceutical quality system. PDA JPST 2005;59:118-26.