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Common Aseptic Validation Protocol

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Table of Contents

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1. Preface

- The document is supplied as a guideline only, not as a guarantee in itself.
- The aim of this protocol is to find an optimum balance between product safety and commercial effort in order to achieve commercial sterility.
- A common aseptic validation protocol defines what can be expected from an aseptic line.
- All the basic definitions this document refers to are explained in the appendix.

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2. Plant Prerequisites

- All relevant equipment operating manuals, recommended maintenance schedules, as well as generally accepted good manufacturing practices etc. are followed.
- The aseptic block must be able to run at nominal speed.
- The upstream units must be able to provide the required quantity and quality of packaging material needed for the microbiological validation.
- The downstream units must be able to handle the required quantity of filled and closed bottles produced during the microbiological validation in a way that ensures the integrity of the sealed bottles.
- To run the validation, the packaging material must meet the correct specifications (see appendix 2).

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3. Validation Format

- Only one format is chosen for microbiological validation: usually the one with the lowest treatment time, i.e., the smallest bottle, because it runs at highest speed and represents the most critical condition.
- In agreement with both parties, also the main contractual format or the one with the most difficult shape or geometry may be selected as validation format.
- If the microbiological performance has been proven with this format, all other formats perform equal or better. In this case, no further microbiological tests with other formats are required.

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4. Low Acid Versus High Acid Products

- This guideline distinguishes between procedures for low acid products and high acid products (according to the FDA definition).

- Low acid products : pH > 4.6
- High acid products : pH ≤ 4.6

- If a low acid validation is passed, the line is also validated for high acid products, if all parameters of the aseptic block remain the same.

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5. Validation Medium

- If the intention is to sell the tested production volume once the aseptic system has been validated, the intrinsic quality of the product must follow the customer's specifications.

- For the microbiological validation of low acid aseptic lines, a culture medium is used to enable visual inspection. Alternatively, the customer’s product may be used, e.g. UHT milk or others.

- For the microbiological validation of high acid aseptic lines,
  a clear product (e.g. apple juice) is used to enable visual inspection.

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6. Thermal Treatment of the Product

- As heat treatment for high acid products is not sufficient for killing all kinds of microorganisms, including heat resistant spores, the temperature/time regime affects the final results of the validation. It is recommended to apply the highest temperature and longest holding time the process unit allows for in order to minimize this influence. For heat treatment explanation, see appendix 3.

- It is recommended to detect thermophilic spore formers in the raw product just before the heat treatment as well as in the aseptic storage tank and in the filled bottles.

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7. Sample Size

The validation is based on three runs of the dedicated numbers of bottles and product.

Low acid products3	High acid products
3 times 10,000 bottles	3 times 30,000 bottles

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8. Validation Sequence

Three consecutive days are chosen to run the tests according to the following sequence:

1st day	2nd day	3rd day
CIP/COP SIP/SOP Run dedicated number of bottles CIP/COP	SIP/SOP Run dedicated number of bottles CIP/COP	SIP/SOP Run dedicated number of bottles CIP/COP

For definitions of CIP/COP/SIP/SOP, see appendix 3

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9. Fill Level of the Bottles

- Half fill the bottles in order to shorten the incubation time because of a higher oxygen
  level in the headspace.
- If bottle handling (transport, stacking) is critical, the fill level may be increased up to the necessary value, e.g., 80% of the nominal volume.
- If nitrogen is used in commercial production, the validation should also include nitrogen dosing.
- If the customer wants to have sellable product, the bottles may be filled with product up to the nominal volume.

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10. Incubation Time and Temperature

Product \ Fill level	Half filled bottles with or without liquid nitrogen	80 % filled bottles with or without liquid nitrogen	Completely filled bottles without liquid nitrogen	Completely filled bottles with liquid nitrogen
Low acid	7 days of incubation @ 30-32° C	7 days of incubation @ 30-32° C	7 days of incubation @ 30-32° C	7 days of incubation @ 30-32° C
High acid	14 days of incubation @ 25-30° C	14 days of incubation @ 25-30° C	14 days of incubation @ 25-30° C	21 days of incubation @ 25-30° C

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11. Storage Conditions (for Low Acid Products)

a) For low acid products

- In case of culture medium or clear product in clear bottles, all 30,000 bottles are visually inspected after 7 days of incubation at a temperature of 30˚ –32˚ C.
- In case of turbid product in clear bottles, all 30,000 bottles are visually inspected after 7 days of incubation at a temperature of 30˚ –32˚ C. Additionally 1,000 bottles per run are checked for pH.
- In case of non-transparent bottles, 30,000 bottles are visually inspected after 7 days of incubation at a temperature of 30˚ –32˚ C. Defective bottles change their size or their shape (gas production or vacuum generation). Additionally 3,000 bottles per run are checked for pH level.

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11. Storage Conditions (for High Acid Products)

b) For high acid products

- Half filled or completely filled bottles are stacked on pallets and stored in a secured place at constant temperature of 25 to 30° C for 14 days. All 90,000 bottles are visually inspected.
- Half filled bottles with liquid nitrogen treatment are stored at constant temperature of 25 to 30° C for 14 days. All 90,000 bottles are visually inspected.
- Completely filled bottles with liquid nitrogen treatment are stored at constant temperature of 25 to 30° C for 21 days. All 90,000 bottles are visually inspected.

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12. Inspection

- After storage, all the bottles are visually inspected.
- Only hermetically sealed packages are taken into consideration where the integrity has been assured during all the production steps.

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13. Evaluation

- All packages failing microbiological testing are to be saved for package integrity testing.

- If a contaminated bottle is found, microbiological identification is carried out in order to determine the origin of the contamination. Especially heat resistant spore identification must be carried out in order to exclude microorganisms coming from
the raw material that may have survived thermal processing.

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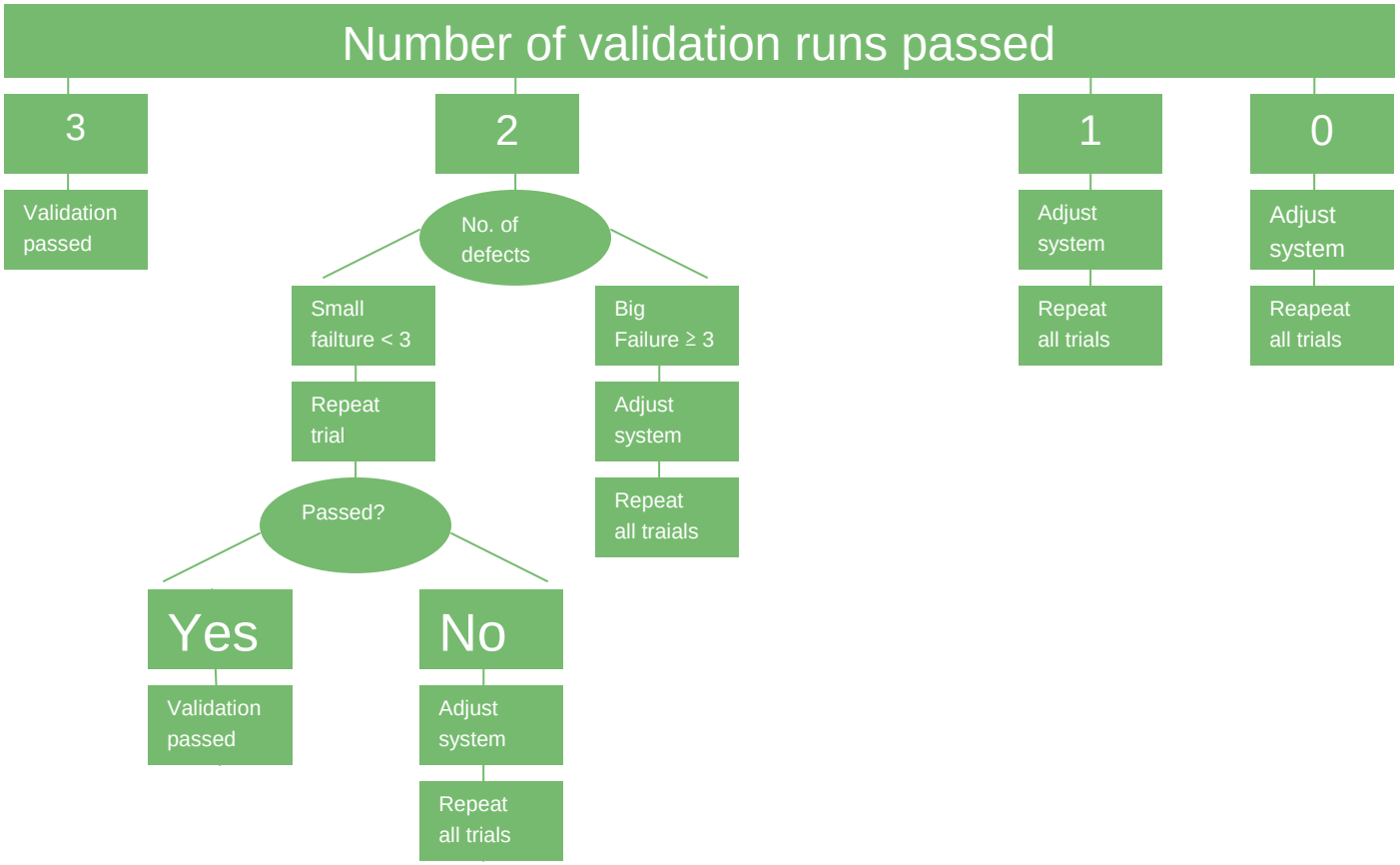
14. Acceptance Criterion

Acceptance Criterion

- The test is accepted if not more than one defective bottle per run is identified.
- Statistically, zero is not defined. This is the reason why zero defects out of a certain amount of bottles can not be proven.

15. Acceptance Steps



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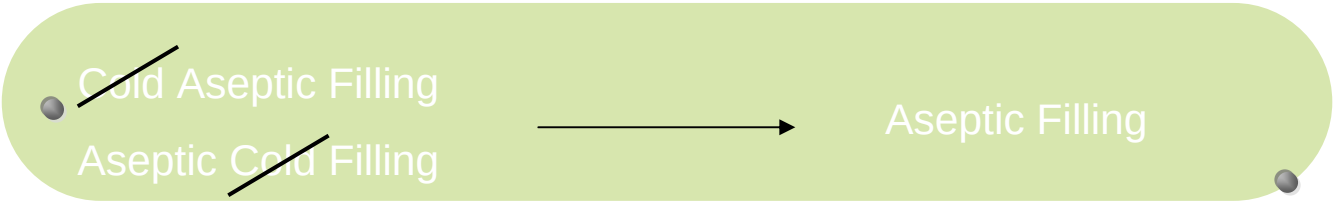
Appendix

Appendix 1: Aseptic Filling Definitions

We agree on aseptic filling in contrast to hot filling and call it

Aseptic filling



This technology is the technical precondition which allows aseptic filling of microbiologically sensitive beverages.

In the process, a pasteurised, sterilised or aseptically filtered product is filled contamination-free into sterilised containers and sealed.

Finished packs sealed according to this procedure have permanent microbiological stability.

Appendix 2: Initial Bioburden of Packaging Material

Microbiologic raw packaging material evaluation:

Before using packaging materials such as preforms, caps or bottles, the initial bioburden will be evaluated. This evaluation will be carried out on 10 units of the packaging material sampled in accordance with good laboratory practice. Preforms or caps will be placed in sterile vials filled with a sterile recovery liquid based on pure sterile water containing 1 ‰ of Tween 80.

Inside the bottles:

Fill 100 ml of the recovery agent in the bottle and close it with a sterile cap.

Complete bottles:

cut the bottles in different parts under aseptic conditions in order to be able to place the pieces in a sterile container. Then proceed as with caps or preforms. After shaking, the recovery liquid is filtered on a membrane that will be incubated on a Plate Count Agar for 5 days at 30° C.After incubation, the CFUs will be counted.

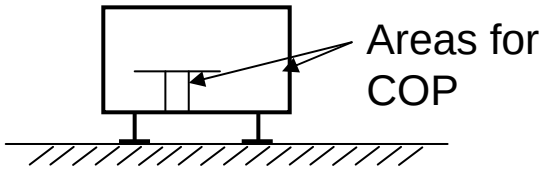
Type of packaging material	Total CFUs	Total CFUs
Complete preforms	Average < 10	Maximum: 50
Complete caps	Average < 10	Maximum: 50
Inside bottle	Average < 10	Maximum: 50
Complete bottle	Average < 20	Maximum: 100

Appendix 3: CIP/COP and SIP/SOP Definition

CIP = cleaning in place

CIP means automatic cleaning of internal parts of pipes, vessels, etc. by means of liquid products (e.g. appropriate chemicals)

COP means to us: cleaning of external surfaces inside an isolator by means of liquid products (appropriate chemicals)



C = Cleaning
S = Sterilization
I = Inside the pipes and vessels
O = Outside the pipes and vessels, but still inside the aseptic chamber
P = Place

SIP = sterilization in place

SIP means automatic sterilization of internal parts of pipes, vessels etc. by means of appropriate methods

SOP = automatic sterilization of external surfaces inside an isolator by means of sterilants, disinfectants or other appropriate methods

Appendix 4: Integrity Test Packaging Material

Procedure using electrical conductivity

The packaging tightness test is performed to detect potential leaks on bottles closed with caps or aluminium foils by means of measurement of electrical resistance.

Equipment, instruments and reagents used

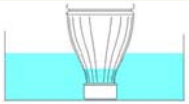
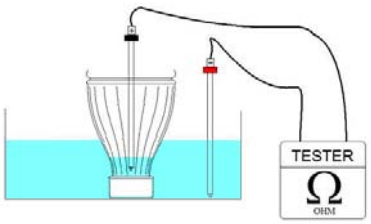
- City water with a measurable conductivity
- Tester for measuring the resistance. The apparatus must reach 2000 MΩ.

Prerequisites

- Define the quantity of bottles required for performing the test (at least 100).
- Define the cap application torque using a specific dynamometer.
- Each bottle must be cut without damaging the closure.

Appendix 4: Integrity Test Packaging Material

Procedure

Each bottle must be cut and soaked in standard city water.	
Fill each bottle with approximately 100 ml of the same standard city water.	
Use a tester, set the resistance measurement modality to 2000 MΩ and insert one of the electrodes into the bottle in contact with the city water and the other one into the bath outside of the bottle. Since the two environments - the one inside and the one outside the bottle - are separated, the tester shows an infinite resistance called “not readable/out of range”. The first measurement must be carried out 10 minutes after start. The bottles remain in the bath for 72 hours. During this period, some measurements are performed before the bottles are removed.	
If the tester shows a readable value, there is a connection between the inside and the outside of the bottle. This is considered an untight package.	No leak is accepted in this trial of 100 bottles

Appendix 5: Inoculation Methods for Packaging Material

Preparation of spore suspension

Inoculation methods for packaging material need to be carried out professionally in order to achieve indisputable results.

General considerations regarding spores

- Bacteria or mould spores should come from a germ which is listed in an official type culture collection and commercially available.
- Different, independent spore suspensions suppliers should be available.
- The spores need to be prepared in a water suspension free from salts and organic material that could provide a residue during drying.
- Spore dilutions need to be prepared in pure sterile water. No Ringer's solution may be used.
- Mould spores need to be free from organic cultivation material. Normally, the spore suspensions are washed 3 to 4 times and centrifuged in order to remove any kind of foreign matter.

Appendix 5: Inoculation Methods for Packaging Material

There are three different methods for the inoculation of the packaging material:

Single-dot inoculation:

The dot has a volume of 10 µl and is placed on the surface of the material to be tested with a micropipette. When starting from a defined concentrated spore suspension (e.g. 10^8 spores/ml), dilutions will be prepared in order to obtain the final correct concentration level of spores. The inoculation area will be marked with a circle.

Multi-dot inoculation:

The dots will have a total volume of 10 µl and are placed on the surface of the material to be tested with a micropipette. When starting from a defined concentrated spore suspension (e.g., 10^8 spores/ml), dilutions will be prepared in order to obtain the final correct concentration level of spores. The sum of the individual dots concentration will be equal to the concentration level that is required. If the final level is 10,000 spores and 10 dots are placed on the material, each dot will have an amount of 1,000 spores. The inoculation area will be marked with circles.

Spray inoculation:

A sprayer that delivers a constant volume and a homogeneous fine film will be used to inoculate the inside of the packaging material. The difficulty is the homogenous distribution
of the inoculant. Operator training is required.

Appendix 6: Test Spores

- Spores of Bacillus atrophaeus ATCC 9372 are recommended for PAA and H_2O_2 systems. More used for low acid products and surface decontamination trials.

- Spores of Aspergillus niger ATCC 16404 are recommended for PAA, H_2O_2 and UV-light systems. Mostly used as the mould reference in the industry. More dedicated for high acid products.

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Glossary

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Glossary Aseptic Terminology

Aseptic process	From Greek, meaning "uncontaminated". An aseptic process which produces a shelf stable product free from microbial contamination to allow distribution without any further usage of chemical preservatives.
Ceaning	Removal of macroscopically identifiable contamination such as food residues, deposits, dust, etc. Proper cleaning eliminates the nutritive medium for microorganisms and is a prerequisite for proper disinfection or sterilization.
Commercial sterility	Commercial sterility of equipment and containers used for aseptic processing and packaging of food means the condition achieved by application of heat, chemical sterilant(s), or other appropriate treatment that renders the equipment and containers free of viable microorganisms capable of reproducing in the food under normal non-refrigerated conditions of storage and distribution. (Source: FDA)

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Glossary Aseptic Terminology

Contamination	Product, media, or surfaces are contaminated if contaminants (including microbes) can be identified on or in them. The term contamination describes the addition of contaminants.
Disinfection	Inactivation of all pathogenic and product-damaging microbes to a level that complies with the respective hygiene requirements.
Germs	Germs are microorganisms and their spores that can multiply under the current, unchanged conditions (i.e. "germinate").
Germ-free	A product is germ-free, if it does not contain any microorganisms that are viable in the respective product (also, beverage-sterile, commercially sterile).

Glossary Aseptic Terminology

Microbiological Contaminants	Contaminants are defined as microbes present at a particular location or within a medium where they are undesired at a particular time.
Microbiologic cal spoilage	Sum of negative, visually and sensorically identifiable changes in food, caused by multiplication of microorganisms and their metabolic activity.
Microorganisms	Here: independently viable organisms that can be classified into the following three groups: bacteria, moulds, yeasts.

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Glossary Aseptic Terminology

Pasteurisation	The act or process of moderately heating a beverage to a specific temperature for a specific period of time in order to kill microorganisms that could cause disease, spoilage, or undesired fermentation.
Recovery rate	The recovery rate has regard to methodical errors and indicates the mean value of microorganisms of the applied initial load which can be recovered in those packages which did not undergo any sterilization treatment.
Sterile	Products, media, or surfaces are sterile if no viable microorganisms can be identified.
Sterilisation	Destruction or removal of microorganisms, including bacterial spores, present in food products or on surfaces.