



PHARMACEUTICAL INSPECTION CONVENTION
PHARMACEUTICAL INSPECTION CO-OPERATION SCHEME

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RECOMMENDATION
ON THE

VALIDATION OF ASEPTIC PROCESSES

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1. DOCUMENT HISTORY

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2. INTRODUCTION

2.1 Purpose

2.1.1 The aim of this document is to provide guidance to the current practice in this field by giving recommendations for the validation of aseptic processes. In particular, the document should provide guidance for GMP inspectors both for training purposes and in preparation for inspections of company premises.

2.2 Scope

2.2.1 This document applies to all manufacturers involved in aseptic processing of finished dosage forms (human and veterinary) as well as manufacturers of sterile labelled bulk drug substances (active pharmaceutical ingredients).

2.2.2 At the time of issue this document reflected the current state of the art. It is not intended to be a barrier to technical innovation or the pursuit of excellence. The advice in this recommendation is not mandatory for industry. However, industry should consider this recommendation as appropriate.

2.3 General information

2.3.1 The basic principles and application of process validation are described in Annex 15 to the EU/PIC/S Guide to GMP and are further elaborated in PIC/S Document PI 006 (Recommendations on Validation Master Plan, Installation and Operational Qualification, Non-Sterile Process Validation, Cleaning Validation) and apply also to aseptic processing. Annex I to the EU/ PIC/S Guide to GMP provides the basic requirements for the manufacture of sterile products including those aseptically processed. The Annex includes requirements, standards and recommendations, for example, for monitoring of the environment and of personnel.

2.3.2 Validation of aseptic processes relies upon prospective, concurrent and retrospective validation as well as re-validation.

2.3.3 *Prospective studies* would include installation and operational qualification for a new or renovated facility as well as product simulation studies and a prospective process validation with the original product according to PIC/S Document PI 006.

- 2.3.4 *Concurrent validation* includes a process validation with the same requirements as for prospective studies, but performed during routine production on qualified equipment.
- 2.3.5 *Retrospective validation* uses the data of earlier manufactures, but is not a recommended technique for aseptic processes.
- 2.3.6 *Re-validation* includes:
- Regular performance of process simulation studies
 - Monitoring of environment, disinfection procedures, equipment cleaning and sterilisation (including containers and closures)
 - Routine maintenance and re-qualification of equipment, e.g. autoclaves, ovens, HVAC (heating, ventilation and air conditioning) systems, water systems, etc.
 - Regular integrity testing of product filters, containers, closures and vent filters
 - Re-validation after changes
- 2.3.7 It is the sum total of all validation data that provides the necessary level of assurance for aseptically produced products.
- 2.3.8 Process simulation studies (media fills) are simulating the whole process in order to evaluate the sterility confidence of the process. Process simulation studies include formulation (compounding), filtration and filling with suitable media. Simulations are made to ensure that the regular process for commercial batches repeatedly and reliably produces the finished product of the required quality. However, each process simulation trial is unique and so it is not possible to extrapolate these results directly to actual production contamination rates.
- 2.3.9 The methods for simulating an aseptic process vary according to the process used for the various types of products, i.e. liquid, semi-liquid and solid dosage forms.
- 2.3.10 In these Recommendations the term “should” indicates requirements that are expected to apply unless shown to be inapplicable or replaced by an alternative demonstrated to provide at least an equivalent level of quality assurance.

3. DEFINITIONS

Action level: Established criteria, e.g. microbial or particulate levels, requiring immediate follow-up and corrective action if exceeded.

Alert limits (environmental monitoring): Established microbial or particulate levels giving early warning of potential drift from normal operating conditions which are not necessarily grounds for definitive corrective action but which require follow-up investigation.

Alert limits (media fill): Established levels or numbers of positive media filled units, the cause of which should be investigated, but which are not necessarily grounds for definitive corrective action.

Aseptic filling: Operation whereby the product is sterilised separately, then filled and packaged using sterilised containers and closures in critical processing zones.

Bioburden: Total number of viable microorganisms on or in pharmaceutical product prior to sterilisation.

Compounding: A process wherein bulk drug substance is combined with another bulk drug substance and/or one or more excipients to produce a drug product.

Environmental monitoring programme: Defined documented programme which describes the routine particulate and microbiological monitoring of processing and manufacturing areas, and includes a corrective action plan when action levels are exceeded.

Growth promotion test: Test performed to demonstrate that media will support microbial growth.

High efficiency particulate air (HEPA) filter: Retentive matrix designed to remove a defined percentage of particulate matter of a defined size.

HVAC: Heating, ventilation and air conditioning

Integrity test: Test to determine the functional performance of a filter system.

Media fills: Method of evaluating an aseptic process using a microbial growth medium. (Media fills are understood to be synonymous to simulated product fills, broth trials, broth fills etc.).

Sampling frequency: Established period for collecting samples.

Shift: Scheduled periods of work or production, usually less than 12 hours in length, staffed by alternating groups of workers.

Sterile: Free of any viable organisms. (In practice, no such absolute statement regarding the absence of microorganisms can be proven, see *sterilisation*.)

Sterilisation: Validated process used to render a product free of viable organisms. Note: In a sterilisation process, the nature of microbiological death of reduction is described by an exponential function.

Therefore, the number of microorganisms which survive a sterilisation process can be expressed in terms of probability. While the probability may be reduced to a very low number, it can never be reduced to zero.

Sterility assurance level (SAL): Probability that a batch of product is sterile. (SAL is expressed as 10^{-n}).

Sterility test: Test performed to determine if viable microorganisms are present.

Vent filter: Non-shedding porous material capable of removing viable and non-viable particles from gases passing in and out of a closed vessel.

4. PROCESS SIMULATION TEST PROCEDURES

4.1 General Comments

4.1.1 The media fill should emulate the regular product fill situation in terms of equipment, processes, personnel involved and time taken for filling as well as for holding.

4.1.2 Where filling takes place over extended periods, i.e. longer than 24 hours, the process simulation test should extend over the whole of the standard filling period. In order to prevent excessively high numbers of units being filled it is usually acceptable to just run the machine for a reasonable time, if the validity of the simulation is not diminished by this procedure.

4.1.3 It should be considered that inert gases will prevent the growth of aerobic microorganisms. Therefore for process simulations sterile filtered air should be used instead of inert gases, also for breaking a vacuum. Where anaerobes are detected in the environmental monitoring or sterility testing, the use of an inert gas should be considered for a process simulation, as inert gas is supporting the growth of anaerobes.

4.1.4 Before enumerating the different process simulation test procedures some preliminary explanations are necessary for the *preparation of liquid media* as it is used for the majority of the process simulation tests. Where a liquid nutrient medium is used it should be prepared in a similar manner to the product. The medium should be dissolved in Water for Injection in a standard manufacturing vessel. If heat is required to dissolve it then only minimal heat should be used. The pH of the medium should be measured and, if necessary, adjusted to bring it into the required range. The medium should be aseptically filtered into an aseptic holding vessel using the normal production filter and processing procedure. In justified cases it may be also acceptable to sterilise the media. All aseptic holding vessels should be covered by a process simulation test on a regular basis unless a validated, pressure hold or vacuum hold test is routinely performed.

4.1.5 The following chapter illustrates the test procedures for the various simulation tests for aseptically produced solutions, lyophiles, suspensions, ointments and powders and summarises the considerations to be made.

4.2 Liquid Products

4.2.1 Vial Products

4.2.1.1 The liquid growth medium for the simulation test is prepared as above and kept in a sterile holding vessel for the maximum permitted holding time before starting the simulation test. If the bulk solution is stored under refrigerated

conditions during the holding time then this should also be performed for the medium. Vials and closures should be prepared as in regular production.

4.2.2 Sterile Products in Plastic Containers

4.2.2.1 Ear and eye drops are typically marketed in plastic containers. Containers, inserts, closures and where applicable overseals are washed and sterilised as in regular production. Instead of sterilisation with heat, irradiation or ethylene oxide are used.

4.2.2.2 Whilst clear plastic containers are frequently used for process simulation trials, the plastic is usually slightly opaque and thus hinders identification of contaminated units that show only a slight haze. In such case examination under natural or room lighting would not suffice. Where opaque containers are used for process simulation trials the whole contents should be removed for examination.

4.2.3 Ampoule Products

4.2.3.1 Open or closed ampoule types may be used. They should be sterilised by dry heat and afterwards used in the simulation test as per the regular production run.

4.2.3.2 Ampoules should be prepared as in regular production.

4.3 Injectable Powder Products

4.3.1 There are two possibilities for simulation of this process. Either by filling a sterilised liquid growth medium into the sterile container or adding a powder (inert or growth medium) before or after a sterile diluent (WFI or growth medium). Inert materials commonly used include: polyethylene glycol 8000 and carboxymethyl cellulose. These materials are usually sterilised by irradiation.

4.4 Suspension Products

4.4.1 This procedure is comparable to the filling of liquid products, except for the process step of maintaining suspension of the ingredients. The stirring or recirculation should be part of the simulation. If aseptic additions are made to the bulk solution these should be simulated by the use of inert sterile liquids/powders.

4.5 Freeze Dried (Lyophilised) Products

4.5.1 Crystallisation of the medium should be prevented because it may reduce the likelihood of recovery of organisms.

4.5.2 Two simulation methods are commonly used. In the first one a dilute medium is subject to a cycle that removes water until a determined medium strength is obtained, but is not subject to freezing. The second method uses full strength medium and requires only a partial vacuum be drawn whilst the chamber should be kept at ambient temperature. There is a risk that the medium may boil over and contaminate the chamber unless conditions are tightly

controlled. The absence of boiling under the defined cycle conditions should be confirmed.

4.6 Semi-Solid Products (e.g. sterile ointments)

4.6.1 For this simulation test the liquid growth medium is thickened to the appropriate viscosity, used as in the routine production procedure. Suitable thickening agents are agar and carboxymethyl cellulose. Other agents would need to be validated with regard to lack of their bacteriostatic and fungistatic properties. Metal and plastic ointment tubes prevent the examination of the medium in-situ. Usually the whole content of the tube should be examined and this is usually achieved by squeezing the contents into a plate (petri dish), and after whirling it is examined for turbidity and fungal colonies under defined light conditions or by performing a sterility test. If properly validated, an alternative method for detection of contamination of semi-solid products could be the use of media which changes colour in the presence of contamination.

4.7 Clinical Trials Materials and Small Batch Size Products

4.7.1 As processes for smaller quantities (less than 3000 units) do not allow an interpretation according to chapter 5 of these Recommendations, any presence of microbial contamination should be regarded as an alert limit. Monitoring and test conditions, like incubation or media selection remain the same as for commercial production runs.

4.7.2 The size of media fills for small batch size products should at least equal the number of containers filled for the commercial product.

4.8 Biological and Biotechnology Products

4.8.1 The manufacture of these products varies, such that there is not one single process. It may be more practical to validate the various segments of the process individually. The frequency of the revalidation should relate to the one of regular, commercial production.

4.9 Sterile Bulk Pharmaceuticals

4.9.1 Whenever possible a growth medium should be used and the process should be simulated as closely as possible to the normal route of manufacturing the sterile bulk drug substance.

4.9.2 The aseptic manufacture of sterile bulk drug substances is a difficult process, which may have numerous individual segments that need to be validated. The possibility of microbial ingress into the system has to be considered after each step of the routine production.

4.9.3 The validation may include segments, where the use of growth media is not feasible.

5. PROCESS SIMULATION TEST CONDITIONS

5.1 Test Performance

- 5.1.1 The process simulation test should follow as closely as possible the routine aseptic manufacturing process and include all critical subsequent manufacturing steps. All equipment should remain the same wherever practicable as for the routine process. Appropriate combinations of container size and opening as well as speed of the processing line should be used (preferably at the extremes).
- 5.1.2 The process simulation test should represent a “worst case” situation and include all manipulations and interventions likely to be represented during a shift.
- 5.1.3 Worst case conditions are often thought to be the largest container with the widest mouth as it is exposed longer to the environment. However, there are exceptions to this and one of them is small ampoules run at the highest speed as the ampoules may be unstable and cause frequent jams thus necessitating frequent operator intervention.
- 5.1.4 The fill volume of the containers should be sufficient to enable contact of all the container-closure seal surfaces when the container is inverted and also sufficient to allow the detection of microbial growth.
- 5.1.5 If batches smaller than 3000 units are produced, the minimum number of containers used for the process simulation should be equal to that of the commercial batch size.
- 5.1.6 Simulation tests should be performed on different days and hours during the week and not only at the beginning of a work day.
- 5.1.7 If the same process is conducted in a separate clean room, this should also be validated.
- 5.1.8 In order to find the possible source of contamination it may be a good advise to video tape the aseptic fill and also number the individual vials or segregate vials in chronological order during incubation.

5.2 Selection of Growth Medium

- 5.2.1 The criteria for the selection of growth medium include: low selectivity, clarity, medium concentration and filterability.
- 5.2.2 *Ability to support growth of a wide range of microorganisms:* The medium should have a low selectivity i.e. be capable of supporting growth of a wide range of microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus niger* and *Clostridium sporogenes* (e.g. Soybean Casein Digest).
- 5.2.3 The selection of the medium has to be based also on the in house flora (e.g. isolates from monitoring etc.).

- 5.2.4 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.
- 5.2.5 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.
- 5.2.6 *Clarity*: The medium should be clear to allow for ease in observing turbidity.
- 5.2.7 *Medium Concentration*: Recommendations of the supplier should be followed unless alternative concentrations are validated to deliver equal results.
- 5.2.8 *Filterability*: If a filter is used in the aseptic manufacturing process, the medium should be capable of being filtered through the same grade as used in production.

5.3 Incubation Conditions

- 5.3.1 It is generally accepted to incubate at 20-25°C for a minimum of 7 days followed immediately, or after a first reading, by incubation at 30-35°C for a total minimum incubation time of 14 days. Other incubation schedules should be based on supporting validation data.
- 5.3.2 Prior to incubation the containers with the microbiological growth medium should be inverted or otherwise manipulated to ensure that all surfaces, included the internal surface of the closure, are thoroughly wetted by the medium. The containers should not be completely filled with medium in order to provide sufficient oxygen for the growth of obligate aerobes. Similarly, containers should not be overlaid with inert gases even though the product may be (see also general comment in Chapter 3.1).
- 5.3.3 The microorganisms present in the containers of the simulation test should be identified to genus but preferably species level to aid determination of the possible sources of the contamination.

5.4 Reading of the Test

- 5.4.1 When inspecting the containers they should be compared to a known sterile container for comparison as some microbial growth shows up as a faint haze which is difficult to detect unless there is a control container to compare against. Personnel should be trained for this task.

5.5 Test Frequency

- 5.5.1 The manufacturer based on his individual circumstances should ultimately decide if more or more frequent tests are required than requested in this chapter.
- 5.5.2 It should be distinguished between “start-up” and “on-going” simulation tests.

- 5.5.3 A “start-up” simulation test consists of three consecutive satisfactory simulation tests per shift and should be carried out before routine manufacturing can start.
- 5.5.4 “Start-up” simulation tests are performed for example for new processes, new equipment or after critical changes of processes, equipment or environment as for example significant personnel changes (a new shift), modifications in equipment directly in contact with the product or modifications in the HVAC system.
- 5.5.5 An “on-going” simulation test consists of one satisfactory simulation test per shift and is mainly performed for the periodic monitoring of aseptic conditions during routine manufacturing but also for example after less critical changes of processes, equipment or environment or if processing lines stand idle for more than 6 months.
- 5.5.6 “On-going” simulation tests should be performed with each shift of each process line at least twice per year under the condition that there were no changes in the normal production procedures and no action limits were exceeded.
- 5.5.7 Exceeding an action level demands a re-validation. Depending on the result of the follow-up investigation this re-validation may require the inclusion of one to three satisfactory process simulation tests.

6. INTERPRETATION OF DATA

- 6.1 After the incubation period of the media-filled containers they are visually examined for microbial growth. Contaminated containers should be examined for evidence of container/closure damage which might compromise the integrity of the packaging system. Damaged containers should not be included as failures (positives) when evaluating results.
- 6.2 The number of containers used for media fills should be sufficient to enable a valid evaluation. For small batches, the number of containers for media fills should at least equal the size of the product batch. The target should be zero growth and the following should apply:
- When filling fewer than 5000 units, no contaminated units should be detected.
 - When filling 5,000 to 10,000 units:
 - a) One (1) contaminated unit should result in an investigation, including consideration of a repeat media fill;
 - b) Two (2) contaminated units are considered cause for revalidation, following investigation.
 - When filling more than 10,000 units:
 - a) One (1) contaminated unit should result in an investigation;
 - b) Two (2) contaminated units are considered cause for revalidation, following investigation.

- 6.3. For any run size, intermittent incidents of microbial contamination may be indicative of low-level contamination that should be investigated. Investigation of gross failures should include the potential impact on the sterility assurance of batches manufactured since the last successful media fill.
- 6.4 All contaminating microorganisms whether or not an alert or action limit has been exceeded should be identified to at least genus and preferably species where practicable to determine the possible source of contamination.
- 6.5 If a process simulation test fails then due account should be taken of products filled between the last successful test and the test failure. Recording of any deviations during the simulation test is important to trace later on the exact cause and to evaluate the consequences. The investigation should identify batches that could be affected during this time period and the disposition of the affected batches should be re-assessed.

7. ENVIRONMENTAL AND PERSONNEL MONITORING

- Annex I of the EU/PIC/S Guide to GMP provides the basis for environmental and personnel monitoring requirements and recommendations.
- Some specific additional guidance is given below on air borne microbial and non-viable particle monitoring, intervention monitoring and staff training.

7.1 Air Borne Microbial and Non-Viable Particle Monitoring

- 7.1.1 It is important to state that the monitoring activity itself should not compromise the product quality. Worst case scenarios of simulations tests should also include monitoring activities.

7.2 Non-viable monitoring

- 7.2.1 The location chosen for monitoring should be checked to ensure that the positions reflect the worst case. For room monitoring, the counts should be performed in locations where there is most operator activity. For the filling environment the counts should be performed adjacent to the filling zone and where components are exposed in such way as to detect operator activity within these areas. Monitoring with sampling probes located in such a way that they monitor the air from the HEPA filter rather than the air immediately surrounding the critical zones should be avoided. However the location of the sample device should not compromise the laminarity of the air flow in the critical zone. Initial validation should be checked to confirm that worst case positions have been adequately identified. These may be reconfirmed during process simulation tests.

7.3 Microbial Monitoring

- 7.3.1 It is usually expected that a combination of the methods identified in the Annex 1 of the EU/PIC/S GMP guide for monitoring microbial levels is used in environmental monitoring programmes where appropriate.

- 7.3.2 Microbial monitoring should be performed in and around areas of high operator activity. It is not unusual to see settle plates and air sample locations well away from such areas. A typical example is where settle plates are located well to the rear of the filling machine where there is little or no operator activity. The same may be true for air sampling. It is important, therefore, to observe operator activity over a period of time and ensure that the monitoring sites are so located as to monitor operator activity.
- 7.3.3 The process simulation test provides an ideal opportunity to confirm that worst case locations have been identified by the use of additional monitoring during the test.
- 7.3.4 A useful monitoring technique is to monitor the filling needles at the end of the filling session.
- 7.3.5 Additional monitoring around the affected area prior to disinfection may provide useful information as to the cause.

7.4 Intervention Monitoring

- 7.4.1 It is essential to include in a simulation test the various interventions that are known to occur during normal production runs, e.g. repair or replacement of needles/tubes, replacement of on-line filters, microbial sampling by monitoring personnel and sampling device, duration of stops on the line, filling and handling of stoppers etc.
- 7.4.2 The process simulation test should last long enough to accommodate all possible interventions and a “worst case scenario”, which may include several unfavourable conditions which are occurring during routine processing.

8. STAFF TRAINING

- 8.1 The routine training of personnel who work in a controlled environment needs special emphasis as people are potentially one of the main sources of micro-organisms in the environment.
- 8.2 Included are not only operators but also other personnel working in a controlled environment as staff responsible for monitoring, equipment maintenance, engineering, washing and preparation.
- 8.3 A formal personnel training programme is needed for all activities in each clean room. This means the programme has to be planned, documented and repeated at adequate intervals to ensure that the once trained individual meets the ongoing requirements for the work in a controlled environment.
- 8.4 This training encompasses subjects like basic microbiology, good manufacturing practice principles, hygiene (disinfection and sanitisation), aseptic connections, alert and action limits, and gowning procedures.
- 8.5 Environmental monitoring personnel need a thorough understanding of the sources of contamination risks (e.g. inadequately disinfected / sterilised sampling equipment) that are involved with the sampling methods.

- 8.6 Periodic process simulation tests (for frequency see Chapter 4.5) are required to ensure that the training of the personnel in charge of filling is effectively maintained.
- 8.7 The competence of an individual should be formally assessed after attending the training courses and active participation of a process simulation test.
- 8.8 The evaluation of filled containers of a simulation test should be done by personnel who are especially trained. They should have routine eye sight tests. This training should include the inspection of filled containers interspersed with contaminated units.
- 8.9 Staff responsible for equipment maintenance, washing and preparation require regular retraining.

9. IMPORTANT FACTORS IN VALIDATION OF ASEPTIC MANUFACTURING

- Beside the elements already described in the previous chapters validation of aseptic manufacturing includes, but is not limited to other important factors described in this chapter.

9.1 Container/Closure Integrity Testing

- 9.1.1 The integrity of particular container/closure configurations should be assured by:
- 9.1.2 Validation of the closure system by filling the container with sterile growth medium and inserting the container in a broth containing approx. 10^6 cfu/ml of a suitable micro-organism. The container is removed after submersion for a recognised period of time, disinfected and then incubated for 14 days. Growth would indicate a failure of the closure system.
- 9.1.3 The container/closure integrity test is normally checked during assessment of the marketing authorisation. The machine set-up is however a critical factor. For vials, 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.

9.2 Container/Closure Sterilisation

- 9.2.1 Problems are rarely encountered with sterilisation of containers. However sterilisation of stoppers might cause problems:
- 9.2.2 Lack of air removal and adequate steam penetration: stoppers should not be packed too densely into trays or bags since this may prevent adequate air removal during the vacuum phase of the autoclave cycle.
- 9.2.3 During the vacuum phases of the autoclave cycle stoppers may clump together to form a tightly bound mass. Pairs of stoppers may become attached to each other with the base of one stopper becoming attached to the top of the other stopper.

9.3 Equipment Cleaning and Sterilisation

9.3.1 Manual cleaning (see PIC/S Document PI 006, Cleaning validation) and sterilisation.

9.3.1.1 Manual cleaning of equipment rarely is a problem but procedures should be checked to ensure that O-rings and gaskets are removed during cleaning otherwise there can be a build up of product residues and/or dirt.

9.3.1.2 If equipment is steam sterilised in an autoclave then the following points should be addressed:

9.3.1.3 Equipment should be wrapped and loaded into the autoclave in such a way to facilitate the removal of air from items in the load.

9.3.1.4 Sterilisation of filters, housings and tubing might cause problems.

9.3.1.5 Problems are usually identified by slow heat up times inside the equipment compared to the chamber temperature. If there is a temperature lag of several minutes then this is usually indicative of entrapped air. The steam will heat up the entrapped air but sterilising conditions will not be obtained as saturated steam will not be present.

9.3.1.6 Only porous load steam autoclaves with a vacuum system to withdraw entrapped air should be used for sterilising equipment.

9.3.1.7 Passive displacement autoclaves (no vacuum to withdraw entrapped air) would normally not be appropriate because of the difficulties in air removal from the load.

9.3.2 Clean-in-place/sterilise-in-place (CIP/SIP).

9.3.2.1 Validation of these systems may be difficult because of the potential incompatibilities in requirements for the design of CIP and SIP facilities. All systems have dead legs to a greater or lesser extent and the required orientation of the dead legs differ for CIP and SIP. The orientation for CIP dead legs is slightly sloping so that the cleaning solution can enter and also drain away. The dead leg for SIP is vertically up so that steam can downwardly displace the air.

9.4 Disinfection

9.4.1 There should be documented procedures describing the preparation and storage of disinfectants and detergents. These agents should be monitored for microbial contamination; dilutions should be kept in previously cleaned containers and should only be stored for defined periods unless sterilised. Disinfectants and detergents used in Grade A and B areas should be sterile at the time of use. If spray bottles are used they should be sterile before being filled and have a short in-use shelf life.

9.4.2 Sporicidal agents should be used wherever possible but particularly for "spraying-in" components and equipment in aseptic areas.

- 9.4.3 The effectiveness of disinfectants and the minimum contact time on different surfaces should be validated.

9.5 Filter Validation

- 9.5.1 Whatever type of filter or combination of filters is used, validation should include microbiological challenges to simulate “worst case” production conditions. The selections of the microorganisms to perform the challenge test (e.g. *P. diminuta*) has to be justified. The nature of the product may affect the filter and so the validation should be performed in the presence of the product. Where the product is bacteriostatic or bacteriocidal an alternative is to perform the test in the presence of the vehicle (product without the drug substance). It may be possible to group similar products and just perform the test on one. The filter integrity test limits should be derived from the filter validation data. The filter manufacturer should also evaluate the maximum permitted pressure differential across the filter and this should be checked against the batch documentation to ensure that it is not exceeded during aseptic filtration.
- 9.5.2 In addition to the validation of the filter type the integrity of each individual product filter used for routine production should be tested before and after use.

9.6 Vent Filters

- 9.6.1 It is important that the integrity of critical gas and air vent filters is confirmed immediately after the filling and if it fails, the disposition of the batch determined. In practice vent filters fail the integrity test more frequently than product filters as generally they are less robust and more sensitive to pressure differentials during steam sterilisation.

9.7 Equipment Maintenance and Testing

- 9.7.1 Aseptic holding and filling vessels should be subject to routine planned preventive maintenance. Gaskets and O-rings should be checked regularly. Sight-glass gaskets are rarely checked routinely and after a number of autoclave cycles may become brittle and allow bypass of room air. All vessels should be subject to regular leak testing (pressure hold or vacuum hold). Where glass vessels are used an alternative leak test method should be devised.
- 9.7.2 Standard Operating Procedures should be checked so as to ensure that any faults or failures of equipment identified by examination, testing or during routine cleaning of equipment are notified immediately to Quality Assurance.

9.8 Blow Fill Seal/Form Fill Seal

- 9.8.1 Where these machines are used for aseptic processing, the following validation aspects should be taken into account:
- 9.8.2 On most machines there are three critical zones: parison formation, parison transfer and the filling zone. An open parison is equivalent to an open container in traditional terms. On most machines only the filling zone is protected by Grade A air showers.

9.8.3 Thermocouples should be placed in those parts of the SIP pipework liable to blockage (steam traps and orifice plates) and where condensate may accumulate (vertically down dead legs). This point however should be dealt with during IQ (Installation Qualification).

9.8.4 Concerning leak testing it has to be considered that some of the techniques are subject to limitations. For example manually performed pressure tests sometimes lack of sensitivity or marginal leakers may not be detected when using the dye bath method, because the use of vacuum post autoclaving will not always detect seam leakers especially at the base of the unit. Therefore a close examination of leaker reject rates is called for. If process simulation leak test rates are significantly higher than production rates this may indicate a higher level of surveillance for the simulation.

9.9 Sterility Test

9.9.1 The sterility test can provide useful information on the validation status of aseptic process. It is important to compare the retest rate for aseptically processed products against that for terminally sterilised products. If aseptically processed products have a higher rate then this may be indicative of sterility problems not identified during validation. This is not an unusual situation as validation cannot take into account all the possible permutations and combinations in equipment, personnel and processes. A typical example of where the sterility test can identify a problem is in the case of damaged O rings on aseptic holding vessels.

9.9.2 However the number of retests should decrease due to the revised Sterility Test in the European Pharmacopoeia. The revision has been made in order to have a harmonised method in the European, the United States and the Japanese Pharmacopoeias. It means that retesting only is allowed if it can be clearly demonstrated that the sterility test was invalid for causes unrelated to the product to be examined. The conditions for considering the method invalid are given in the method. If retesting is allowed it should be made with the same number of containers as in the first test.

9.9.3 Provision should be made to sample a sufficient amount of product from the same location of the load in case of retesting is performed.

10. REVISION HISTORY

Date	Version Number	Reasons for revision
17 April 2000	PE 002-2	Copyright statement inserted
31 July 2001	PI 007-1	Document adopted as a guidance document for inspectors by PIC/S Committee on 22 May 2001. As a result, the document reference number was changed. Other changes: "editor" (cover page), "purpose" (para. 1.1), "scope" (1.2), cross-references to other documents, new page and paragraph numbering.

1 July 2004	PI 007-2	Change in the Editor's co-ordinates
25 September 2007	PI 007-3	Change in the Editor's co-ordinates
7 February 2009	PI 007-4	Amendment of paragraph 5.3.1 (incubation)
6 May 2009	PI 007-5	Revision of section 6 (interpretation of data) based on revised Annex 1 to the PIC/S GMP Guide
