



28 April 2006

To: Ms. Sabine Atzor, European Commission, Brussels  
Mr. David Cockburn, EMEA, London

Via e-mail to: [sabine.atzor@cec.eu.int](mailto:sabine.atzor@cec.eu.int)  
[david.cockburn@emea.eu.int](mailto:david.cockburn@emea.eu.int)

Doc. Ref: EMEA/INS/GMP/318222/2005, GMP Annex 1: Proposals for amendment to the environmental classification table for particles and associated text, amendment to section 42 concerning acceptance criteria for media simulations, amendment to section 52 concerning bio-burden monitoring, and additional guidance in section 88 on the sealing of vials.

Expert members of ISPE have taken the opportunity to review the November 2005 proposals to revise Annex 1 of the EU GMP. We sincerely appreciate the opportunity to contribute to such an important document.

Attached please find a document that provides the assembly of ISPE member's comments. The comments have been collated against the accompanying line-numbered version of the proposed revisions which we trust will aid you in your evaluation. Please note that the format of the comments sheet identifies the issue and suggests, where appropriate, changes that might be made.

ISPE is active in the field of sterile products manufacture and has developed a Baseline® Guide related to sterile products facilities to help amplify the requirements expressed in the GMPs. It is with this in mind that many of the comments seek to achieve reasonable international harmony to help achieve consistent facilities and operations worldwide.

In summary, the ISPE comments suggest the following additional improvements to the modified Annex 1 document:

- Improve the language and clarity of the document to ensure effective international understanding. The importance of this is emphasised by the fact that this revised Annex 1 will be immediately integrated into the PIC/S GMP.
- Make further changes to help harmonize better with ISO 14644-1, which is the internationally accepted standard for non-viable particle classification.
- We agree with the intent of revised Clause 47 to harmonize process simulation tests (media fills) with the equivalent FDA guidance document.



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- We are most concerned about the revisions proposed to the guidance for the handling of uncapped lyophilization and other vials between partial stoppering and final sealing. The revision appears to suggest that the application of the over-seals should be an aseptic process. The new Clause 93 received the largest number of comments. We have included in our comments suggestions to improve and clarify current good practice.

ISPE and its members, through the European HQ in Brussels, would be very pleased to assist the work of the EMEA in completion of this revision of Annex 1 through additional meetings, forums or work sessions. We look forward to a rapid conclusion to this important update of a critical regulatory document.

Yours sincerely,

Robert P. Best  
President & CEO, ISPE

Enclosures: ISPE comments on EU GMP Annex 1 – April 2006  
Line Numbered Doc. Ref: EMEA/INS/GMP/318222/2005/Correction

<p><b>April 2006</b></p> 	<p><b>Proposed Revisions to Annex 1 of the EU GMP</b></p> <p>Comments on EMeA document: EMEA/INS/GMP/318222/2005/Correction</p>	
<p>Reference document:</p>	<p>For clarity, this document uses a line numbered version of the proposed changes, which accompanies these comments.</p>	
<p>Note: these comments refer to the proposed revisions and certain associated matters.</p>	<p>Comments prepared by ISPE on behalf of its members.</p> <p>ISPE Headquarters</p> <p>3109 W. Dr. Martin Luther King Jr. Blvd., Suite 250</p> <p>Tampa, FL 33607</p> <p>USA</p> <p><a href="http://www.ispe.org">www.ispe.org</a></p>	

Clause or section reference	Issue or comment	Proposal to improve
General editing	There are several occurrences within the proposed revisions and the remaining text where phraseology is unclear or inconsistent.	<p>Once the changes have been finalised for the modified sections of Annex 1, it is recommended that the whole Annex is reviewed for consistency of terminology, clarity of meaning and accuracy of references. This should be a technical editing task.</p> <p>Matters of consistency include items such as 5.0 µm instead of 5µm.</p>
Clause 3 Line 19	<p><i>The table is not in accordance with ISO 14644-1. What is meant with the statement “in accordance with...”?</i></p> <p>Clean rooms and clean air devices should be classified in accordance with EN ISO 14644-1.</p>	<p>Cleanrooms and clean air devices should be classified using the methodology found in ISO 14644-1 and applying the limits in table below. It should be noted that this guidance advises certain sample sizes that are not compliant with ISO 14644-1.</p>
Clause 3 Line 21-22	<p><i>The application of the limits can be applied to both classification and monitoring.</i></p> <p>The maximum permitted airborne particle concentration for each grade is given in the following table.</p>	<p>The maximum permitted airborne particle concentration for each grade is given in the following table. These levels can be applied to both classification and monitoring.</p>

Clause or section reference	Issue or comment	Proposal to improve
<p>Clause 3 The table Line 22-23</p>	<p><i>There were many comments about the table.</i></p> <ul style="list-style-type: none"> <li>• <i>Align levels exactly with ISO 14644-1.</i></li> <li>• <i>Remove the <math>\geq 5.0 \mu\text{m}</math> particle size requirement.</i></li> <li>• <i>Create full alignment with FDA's current Aseptic Processing Guidance.</i></li> <li>• <i>Remove the "at rest" requirement.</i></li> <li>• <i>Correct the error for Grade C "in operation" at <math>\geq 0.5 \mu\text{m}</math>.</i></li> </ul>	
<p>Clause 3 Line 23-24 Note under the table</p>	<p><i>This note is unclear.</i></p> <p>* The maximum permitted number of particles at <math>\geq 5.0\mu\text{m}</math> is established at 1/ m<sup>3</sup> but for reasons related to false counts associated with electronic noise, stray light, etc. the limit of 20/m<sup>3</sup> could be considered.</p>	<p>The maximum permitted number of particles at <math>\geq 5.0\mu\text{m}</math> is established at 1/ m<sup>3</sup> since this represents the low levels of contamination that should be achievable in these areas. However, it is recognised false count errors due to electronic noise, shed particles from counting system, stray light, etc. can render accurate measurement unreliable. Subject to justification, a limit of up to 20/m<sup>3</sup> may be considered.</p> <p>Alternatively, the table could be revised with 20 in lieu of 1, and with a note identifying that normally much lower levels should be expected.</p>
<p>Clause 4 Line 25-32</p>	<p><i>This section is confusing and doesn't deal with the requirements of Grade B "at rest." The suggested sampling requirement for Grade B "at rest" is impractically large for large grade B areas that might include corridors and other support accommodation.</i></p> <p><i>Spending a lot of time on the classification of Grade B "at rest"</i></p>	

Clause or section reference	Issue or comment	Proposal to improve
	<p><i>has little value related to the potential for product contamination “in operation.”</i></p> <p><i>The requirement to retest the “at rest” classification should be clarified. It is questioned whether “at rest” classification needs to be undertaken more frequently than at start-up and after significant change or a performance problem.</i></p> <p>For classification purposes, in Grade A zones, a minimum sample volume of 1m<sup>3</sup> should be taken. Grade A and Grade B (at rest) is similar to EN ISO Class 5 for particles &lt;0.5 µm. For classification purposes EN/ISO 14644-1 methodology defines both the minimum number of sample locations and the sample size based on the class limit of the largest considered particle size. It should be noted that this will give rise to a sampling time of about 35 minutes at each location when using a particle counter with a sample rate of 28.3 litre/minute (one cubic-foot per minute).</p>	<p>Air cleanliness classification methodology is specified in EN/ISO 14644. It defines the minimum number of sample locations, the sample size based on the class limit of the largest considered particle size, and how to evaluate the data collected.</p> <p>If this standard were applied to the 1 particle/m<sup>3</sup> limit for particles &lt;5.0 micron (the largest considered particle size) found in Grade A and B “at rest”, ISO 14644-1 would require a sample size of 20 m<sup>3</sup>. At a typical sample rate of 28.3 l/min, this would take 11.8 hrs to collect at each location. Since this is impractical in some applications, a smaller sample size of 1m<sup>3</sup> at each location is permitted for Grade A (“at rest” and “in operation”).</p> <p>For Grade B “at rest”, the sample size should be based on the &lt;0.5 micron particle size.</p> <p>When using this basis for classification of Grade A and Grade B “at rest”, it should be noted that the &lt;5.0 micron classification can only be reported as indicative because the sample size is insufficient to meet the requirements of ISO 14644-1. For Grade C, the sample size should be based on the standard requirements of ISO 14644-1.</p> <p>It should be noted that a sample size of 1m<sup>3</sup> will give rise to a sampling time of about 35 minutes at each location when using a particle counter</p>

Clause or section reference	Issue or comment	Proposal to improve
		with a sample rate of 28.3 litre/minute (one cubic-foot per minute).
<p>Clause 4 Line 34-38</p>	<p><i>This document specifies “short length of sample tubing.” This is ambiguous and in any case the tubing length should be validated.</i></p> <p>Portable particle counters with a short length of sample tubing should be used for classification purposes because of the relatively higher rate of precipitation of particles &lt;5.0µm particles in remote sampling systems with long lengths of tubing.</p>	
<p>Clause 4 Line 40-43</p>	<p><i>The introduction of the requirement to do “in operation” classification is new. <u>Is this new requirement mandatory?</u></i></p> <p><i>The clause about 14644-2 should be separated and clarified.</i></p> <p>“In operation” classification may be demonstrated during media fills because of the worst-case simulation required for this. EN ISO 14644-2 provides information on testing to demonstrate continued compliance with the assigned cleanliness classifications.</p>	<p>When it is decided to undertake “In operation” classification of a cleanroom or cleanzone, this may be undertaken during normal operations, media fills (process simulation) or other simulated “worst-case” operational state.</p> <p>EN ISO 14644-2 provides information on testing and monitoring to demonstrate continued compliance with the assigned cleanliness classifications.</p>
<p>Lines 50-59</p>	<p><i>Improve clarity of text to ensure monitoring options are clear and that risk-based application is encouraged.</i></p> <p><i>Some members wished that the Grade B monitoring requirement be removed altogether or at least the need for it be determined</i></p>	

Clause or section reference	Issue or comment	Proposal to improve
	<p><i>on the basis of some assessment of contamination potential, risks and the benefits of monitoring.</i></p> <p><i>Improve formatting of text.</i></p> <p>For Grade A zones a continuous or frequent sampling particle monitoring system should be used, except where justified, e.g. the filling of live virus vaccines. It is recommended that a similar system be used for Grade B zones although the sample frequency may be decreased. Such systems may consist of independent particle counters; or have one particle counter that is linked to a number of sampling ports sequentially via a tubing manifold system. Where remote sampling systems are used, the length of tubing and the radii of any bends in the tubing must be validated. The Grade A zone should be monitored at such a frequency that all interventions and other transient events would be captured and alarms triggered if excursions from defined operating norms occur.</p>	<p>For Grade A zones a continuous or frequent sampling particle monitoring system should be used, except where justified by the likelihood of the system becoming unacceptably contaminated from the product or process. The filling of live virus vaccines and powders are examples. The Grade A zone should be monitored at such a frequency that all interventions and other transient events would be captured and alarms triggered if excursions from defined operating norms occur.</p> <p>It is recommended that a similar monitoring system be used for Grade B zones in applications where there is limited separation from the associated Grade A zone, although the sample frequency may be decreased.</p> <p>Particle monitoring systems may consist of an array of independent particle counters, have one particle counter that is linked to a number of sampling ports sequentially via a tubing manifold system, or use a portable particle counter located close to the environment to be monitored.</p> <p>Where remote sampling systems are used, the length of tubing and the radii of any bends in the tubing must be evaluated to demonstrate adequate recovery of particles of the target size(s).</p>
<p>Clause 5 Lines 61-64</p>	<p><i>Clarify this text, better to explain the requirement.</i></p> <p>The sample sizes taken for monitoring purposes using automated systems will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal classification of clean rooms and</p>	<p>The sample sizes taken for monitoring purposes using automated systems will usually be a function of the sampling rate of the system used, the particle size(s) considered and the likely frequency of events that one wishes to monitor.</p>

Clause or section reference	Issue or comment	Proposal to improve
	clean air devices.	It is not necessary for the sample volume to be the same as that used for formal classification of clean rooms and clean air devices. Smaller sample sizes can be taken. It is recommended that both individual samples and cumulative counts over a longer period of time are evaluated.
Clause 6 Line 67-74	<p><i>Improve and clarify the text.</i> <i>Move text up from clause 7 for better continuity.</i></p> <p>In Grade A and B zones, the monitoring of the 5.0 µm particle concentration count takes on a particular significance as it is an important diagnostic tool for early detection of failure. The occasional indication of ≥5.0 µm particle counts may be false counts due to electronic noise, stray light, coincidence, etc. However consecutive or regular counting of low levels is an indicator of a possible contamination event and should be investigated. Such events may indicate early failure of the HVAC system, filling equipment failure or may also be diagnosis of poor practices during machine set-up and routine operation.</p>	<p>In Grade A and B zones, the monitoring of the &lt;5.0 µm particle concentration count has particular significance as it is an important diagnostic tool for early detection of failure.</p> <p>When monitoring environments with very low limits of airborne particle concentration, the occasional indication of a &lt;5.0 µm particle count may be false due to electronic noise, stray light, coincidence, release of particles adhered to the counting system, etc. However consecutive or regular counting of low levels is an indicator of a possible contamination event and should be investigated.</p> <p>Such events may indicate early failure of the HVAC system, filling equipment failure or may also be diagnosis of poor practices during machine set-up and routine operation.</p> <p>It is accepted that it may not always be possible to demonstrate low levels of &lt;5.0 µm particles at the point of fill when filling is in progress, due to the generation of particles or droplets from the product itself.</p>

Clause or section reference	Issue or comment	Proposal to improve
<p>Clause 7 Line 76-81</p>	<p><i>Improve clarity, move text up to clause 6 for better continuity</i></p> <p>The particle limits given in the table for the “at rest” state should be achieved after a short “clean up” period of 15-20 minutes (guidance value) in an unmanned state after completion of operations. It is accepted that it may not always be possible to demonstrate low levels of 5 µm particles at the point of fill when filling is in progress, due to the generation of particles or droplets from the product itself.</p>	<p>The particle limits given in the table for the “at rest” state should be achieved after a short “clean up” period of 15-20 minutes (guidance value) in an unmanned state after completion of operations.</p>
<p>Clause 57 Line 125-135</p>	<p><i>Except for materials at high risk for contamination and/or endotoxin, e.g., of natural or microbial origin, a requirement to do bioburden on each batch before filtration is onerous. Given that the filter validations are done at very high challenge levels (~10<sup>7</sup> per cm<sup>2</sup>) and each filter is integrity tested at each use, what would a pre-filtration bioburden determination accomplish and what would a specification be?</i></p> <p><i>Require only materials at risk to be routinely evaluated for bioburden. All others should be evaluated on a periodic basis.</i></p> <p>The bioburden should be monitored before sterilisation. There should be working limits on contamination immediately before sterilisation, which are related to the efficiency of the method to be used. Bioburden assay should be performed on each batch for both aseptically filled product and terminally sterilised products. Where overkill sterilisation parameters are set for terminally sterilised products, bioburden might be monitored only at suitable scheduled intervals. For parametric release systems, bioburden</p>	

Clause or section reference	Issue or comment	Proposal to improve
	<p>assay should be performed on each batch and considered as an in-process test. Where appropriate the level of endotoxins should be monitored.</p>	
<p>Clause 57 Line 133-135</p>	<p><i>This clause is a problem for suspensions and vaccines that cannot be sterile filtered prior to filling.</i></p> <p>All solutions, in particular large volume infusion fluids, should be passed through a microorganism-retaining filter, if possible sited immediately before filling.</p>	<p>All solutions, in particular large volume infusion fluids, should be passed through a microorganism-retaining filter, if possible sited immediately before filling. When the final medicinal product cannot be sterile-filtered, each component of the formulation must be rendered sterile and mixed aseptically.</p>

Clause or section reference	Issue or comment	Proposal to improve
<p>Clause 93 Line 138-151</p>	<p><i>This text is confusing and suggests that aseptic oversealing might be required.</i></p> <p><i>There is also confusion between handling lyo vials with partially inserted stoppers and handling vials with fully inserted stoppers.</i></p> <p><i>The text order can be improved to clarify.</i></p> <p>Containers should be closed by appropriately validated methods. Containers closed by fusion, e.g. glass or plastic ampoules should be subject to 100% integrity testing. Samples of other containers should be checked for integrity according to appropriate procedures.</p> <p>Partially stoppered freeze drying vials should be maintained under Grade A conditions at all times, from the time of partial stoppering to capping.</p> <p>The container closure system for aseptically filled vials is not fully integral until the aluminium cap has been crimped into place. Vials should be maintained in Grade A environment until the cap has been crimped. As the equipment used to crimp vial caps can generate large quantities of non-viable particulates, the equipment should be located at a separate station equipped with adequate air extraction. The capping station may not be able to meet Grade A conditions for non-viable particles in the “in operation” condition but should meet the microbiological requirements.</p>	<p>Containers should be closed by appropriately validated methods. Containers closed by fusion, e.g. glass or plastic ampoules should be subject to 100% integrity testing. The container closure system for filled vials is not fully integral until the aluminium cap has been crimped into place. Samples of other containers should be checked for integrity according to appropriate procedures.</p> <p>Partially stoppered freeze drying vials should be maintained under Grade A conditions at all times, from the time of partial stoppering to the point of leaving the aseptic processing area.</p> <p>As the equipment and process associated with handling and crimping vial caps can generate large quantities of particulates, the equipment should be separated from where containers are open to prevent ingress of contamination. If air extraction is provided to remove the particles, this must not adversely influence the Grade A protection. For aseptic processing, it may also be considered advantageous to locate the</p>

Clause or section reference	Issue or comment	Proposal to improve
		<p>capping/over-sealing outside the aseptic processing area. This avoids the need to sterilise the over-seals and apply them aseptically.</p> <p>If stoppered vials exit an aseptic processing area (Grade B) prior to capping, appropriate assurances should be put in place to safeguard the product until completion of the crimping step. The use of devices for on-line detection of improperly seated stoppers can provide additional assurance. The containers should be protected by a Grade A environment within a Grade B background up to the point of leaving the aseptic processing room or zone. The transfer to the capping/over-sealing station should be protected with ISO 5 A air supply, be enclosed and configured to prevent operator intervention, and be located in a surrounding environment of at least Grade D. It should be noted that the capping/over-sealing station may not be able to meet Grade A conditions and that the overseals will not be sterile.</p>
Section 3	<p><i>This text is confusing. It is recognised that it is not part of the proposed changes, but this text really needs clarification to meet industry requirements and current practice.</i></p> <p>Grade A : The local zone for high risk operations, e.g. filling zone, stopper bowls, open ampoules and vials, making aseptic connections. Normally such conditions are provided by a laminar air flow work station. Laminar air flow systems should provide a homogeneous air speed in a range of 0.36 – 0.54 m/s (guidance</p>	<p>Grade A : The local zone for high risk operations, e.g. filling zone, stopper bowls, open ampoules and vials, making aseptic connections. Normally such conditions are provided by a unidirectional airflow work-station.</p>

Clause or section reference	Issue or comment	Proposal to improve
	<p>value) at the working position in open clean room applications. The maintenance of laminarity should be demonstrated and validated.</p> <p>A uni-directional airflow and lower velocities may be used in closed isolators and glove boxes.</p>	<p>Unidirectional airflow systems should provide a homogeneous airflow over the critical zone working level. An average air speed of 0.45 m/s +/- 20% (guidance value) at the filter level is appropriate in open clean room applications. EN/ISO 14644-3 provides a specification and information about filter level and working level air velocity measurement.</p> <p>The maintenance of uniformity should be demonstrated by airflow visualisation.</p> <p>Non-unidirectional airflow and lower unidirectional airflow velocities may be used in closed isolators and glove boxes.</p>
End of comments		



London, 21 September 2005

Doc. Ref: EMEA/INS/GMP/318222/2005/Correction

**GMP Annex 1: Proposals for amendment to the environmental classification table for particles and associated text, amendment to section 42 concerning acceptance criteria for media simulations, amendment to section 52 concerning bio-burden monitoring, and additional guidance in section 88 on the sealing of vials.**

*The existing text of clause 3 remains unchanged up to and including the following:*

Grade C and D: Clean areas for carrying out less critical stages in the manufacture of sterile products.

*The remaining text of clause 3 up to but not including the following:*

Other characteristics such as temperature and relative humidity depend on the product and nature of the operations carried out. These parameters should not interfere with the defined cleanliness standard.

*and clause 4 is replaced by:*

Clean room and clean air device classification

Clean rooms and clean air devices should be classified in accordance with EN ISO 14644-1. Classification should be clearly differentiated from operational process environmental monitoring. The maximum permitted airborne particle concentration for each grade is given in the following table.

Grade	at rest		in operation	
	Maximum permitted number of particles/m <sup>3</sup> equal to or above			
	0.5 µm	5.0µm	0.5 µm	5.0µm
A	3 500	1*	3 500	1*
B	3 500	1*	350 000	2 000
C	350 000	2 000	3 5000 000	20 000
D	3 500 000	20 000	Not defined	Not defined

\* The maximum permitted number of particles at  $\geq 5.0\mu\text{m}$  is established at  $1/\text{m}^3$  but for reasons related to false counts associated with electronic noise, stray light, etc. a limit of  $20/\text{m}^3$  could be considered.

For classification purposes, in Grade A zones, a minimum sample volume of  $1\text{m}^3$  should be taken. Grade A and Grade B (at rest) is similar to EN ISO Class 5 for particles  $\geq 0.5\mu\text{m}$ . For classification purposes EN/ISO 14644-1 methodology defines both the minimum number of sample locations and the sample size based on the class limit of the largest considered particle size. It should be noted that this will give rise to a sampling time of about 35 minutes at each location when using a particle counter with a sample rate of 28.3 litre/minute (one cubic-foot per minute).

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Portable particle counters with a short length of sample tubing should be used for classification purposes because of the relatively higher rate of precipitation of particles  $\geq 5.0\mu\text{m}$  particles in remote sampling systems with long lengths of tubing. Isokinetic sample heads shall be used in unidirectional airflow systems.

“In operation” classification may be demonstrated during media fills because of the worst-case simulation required for this.  
EN ISO 14644-2 provides information on testing to demonstrate continued compliance with the assigned cleanliness classifications.

5. Clean room and clean air device monitoring

Clean rooms and clean air devices should be routinely monitored in operation and the monitoring locations based on formal risk analysis study and results obtained during the initial classification of rooms and/or devices.

For Grade A zones a continuous or frequent sampling particle monitoring system should be used, except where justified, e.g. the filling of live virus vaccines. It is recommended that a similar system be used for Grade B zones although the sample frequency may be decreased. Such systems may consist of independent particle counters; or have one particle counter that is linked to a number of sampling ports sequentially via a tubing manifold system. Where remote sampling systems are used, the length of tubing and the radii of any bends in the tubing must be validated. The Grade A zone should be monitored at such a frequency that all interventions and other transient events would be captured and alarms triggered if excursions from defined operating norms occur.

The sample sizes taken for monitoring purposes using automated systems will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal classification of clean rooms and clean air devices.

6 In Grade A and B zones, the monitoring of the  $5.0\mu\text{m}$  particle concentration count takes on a particular significance as it is an important diagnostic tool for early detection of failure. The occasional indication of  $\mu\text{m}$  particle counts  $\geq 5.0\mu\text{m}$  may be false counts due to electronic noise, stray light, coincidence, etc. However consecutive or regular counting of low levels is an indicator of a possible contamination event and should be investigated. Such events may indicate early failure of the HVAC system, filling equipment failure or may also be diagnosis of poor practices during machine set-up and routine operation.

7 The particle limits given in the table for the “at rest” state should be achieved after a short “clean up” period of 15-20 minutes (guidance value) in an unmanned state after completion of operations. It is accepted that it may not always be possible to demonstrate low levels of particles  $\geq 5\mu\text{m}$  at the point of fill when filling is in progress, due to the generation of particles or droplets from the product itself.

82 8 For Grade D areas in operation, the requirements and limits will depend on the  
83 nature of the operations carried out, but the recommended “clean up period”  
84 should be attained.

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86 *The new clause 9 takes up the existing text of clause 3 at the following point:*

87 Other characteristics such as temperature and relative humidity depend on the  
88 product and nature of the operations carried out. These parameters should not  
89 interfere with the defined cleanliness standard.

90 *The existing clauses from 5 to 41 are unchanged but re-numbered as 10-46.*  
91 *Clause 47 (formerly clause 42) is changed as follows:*

92 47. Validation of aseptic processing should include a process simulation test using a  
93 nutrient medium (media fill). Selection of the nutrient medium should be made  
94 based on dosage form of the product and selectivity, clarity, concentration and  
95 suitability for sterilisation of the nutrient medium. The process simulation test  
96 should imitate as closely as possible the routine aseptic manufacturing process  
97 and include all the critical subsequent manufacturing steps. It should also take  
98 into account various interventions known to occur during normal production as  
99 well as worst-case situations. Process simulation tests should be performed as  
100 initial validation with three consecutive satisfactory simulation tests per shift and  
101 repeated at defined intervals and after any significant modification to the  
102 HVAC-system, equipment, process and number of shifts. Normally process  
103 simulation tests should be repeated twice a year per shift and process. The  
104 number of containers used for media fills should be sufficient to enable a valid  
105 evaluation. For small batches, the number of containers for media fills should at  
106 least equal the size of the product batch. The target should be zero growth and the  
107 following recommendations apply:

- 108  
109 i When filling fewer than 5000 units, no contaminated units should be  
110 detected.  
111 ii When filling 5,000 to 10,000 units: 1 contaminated unit should result in  
112 an investigation, including consideration of a repeat media fill. 2  
113 contaminated units are considered cause for revalidation, following  
114 investigation.  
115 iii When filling more than 10,000 units:  
116 1 contaminated unit should result in an investigation.  
117 2 contaminated units are considered cause for revalidation, following  
118 investigation.  
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120 Investigation of gross failures should include the potential impact on the sterility  
121 assurance of batches manufactured since the last successful media fill.  
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123 *Clauses 43 to 51 are unchanged but re-numbered as 48-56. Clause 57 (formerly*  
124 *clause 52) is changed as follows:*

125 57. The bioburden should be monitored before sterilisation. There should be working  
126 limits on contamination immediately before sterilisation, which are related to the  
127 efficiency of the method to be used. Bioburden assay should be performed on  
128 each batch for both aseptically filled product and terminally sterilised products.  
129 Where overkill sterilisation parameters are set for terminally sterilised products,  
130 bioburden might be monitored only at suitable scheduled intervals. For  
131 parametric release systems, bioburden assay should be performed on each batch  
132 and considered as an in-process test. Where appropriate the level of endotoxins  
133 should be monitored. All solutions, in particular large volume infusion fluids,  
134 should be passed through a microorganism-retaining filter, if possible sited  
135 immediately before filling.

136 *Clauses 53 to 87 are unchanged but re-numbered as 58-92. Clause 93 (formerly*  
137 *clause 88) is changed as follows:*

138 93. Containers should be closed by appropriately validated methods. Containers  
139 closed by fusion, e.g. glass or plastic ampoules should be subject to 100%  
140 integrity testing. Samples of other containers should be checked for integrity  
141 according to appropriate procedures.

142 Partially stoppered freeze drying vials should be maintained under Grade A  
143 conditions at all times, from the time of partial stoppering to capping.

144 The container closure system for aseptically filled vials is not fully integral until  
145 the aluminium cap has been crimped into place. Vials should be maintained in  
146 Grade A environment until the cap has been crimped. As the equipment used to  
147 crimp vial caps can generate large quantities of non-viable particulates, the  
148 equipment should be located at a separate station equipped with adequate air  
149 extraction. The capping station may not be able to meet Grade A conditions for  
150 non-viable particles in the “in operation” condition but should meet the  
151 microbiological requirements.

152 *Clauses 89 to 93 are unchanged but re-numbered as 94-98.*