Author: Tim Sandle
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CLEANROOMS AND ASSOCIATED CONTROLLED ENVIRONMENTS - BIOCONTAMINATION CONTROL: DISSECTING THE STANDARD

INTRODUCTION

In 2020 a new contamination control standard has been issued, specifically focusing on biocontamination control, titled EN 17141: 2020 "Cleanrooms and associated controlled environments — Biocontamination control" (1). The standard has been long awaited and this author has been lobbying for such a standard for a decade or so. However, the final form, while containing much of value, is flawed in places.

Biocontamination control is generally concerned with microorganisms, viruses, microbial toxins, and particulates (2). However, in relation to this definition, the scope of the new standard limits the definition of contamination to bacteria and fungi (or what are described as 'mould and yeast' in the standard). While viruses and prions are a relatively specialist area, it is immediately disappointing that endotoxin does not feature in the scope of the document. The standard seeks to replace the current international two-part contamination control standard ISO 14698 Parts 1 and 2.

This article assesses the content of EN 17141: 2020 and discusses the points of importance, focusing on those which add value to those who are tasked with ensuring effective biocontamination control, as well as describing some areas of ambiguity, contradiction, and potential inaccuracy. These latter issues, in the context of pharmaceuticals and healthcare, appear to arise due to a lack of input from pharmaceutical microbiology subject matter experts. The standard applies to four industrial sectors, although the focus of this article is with sterile products and medical devices (hospital environments and food processing will not be covered in any detail). This review is undertaken from a pharmaceutical microbiology perspective, based on the experience of the author.

EN 17141: Evolution and Its Relationship to ISO 14698

As indicated above, the inference with EN 17141: 2020 is that it is intended to replace the 2003 issued ISO 14698 standard (3). However, according to the status on the International Standard Organization's website, ISO 14698 has not (yet?) been withdrawn as an official standard.

Furthermore, from the pharmaceutical and healthcare products perspective, the scope of the 'EN' standard will be for products manufactured and (potentially) imported into the European Union. However, the standard does not, at this stage, feature in EU GMP Annex 1 which is currently undergoing a revision (as reviewed by this author for the *Journal of GxP Compliance*) (4).

The drafting of the standard fell under the committee CEN/TC 243/WG 5 - Biocontamination control, which reports into the committee CEN/TC 243 - Cleanroom technology. EN 17141: 2020 was published on 12th August 2020. The standard went through two drafts, issued in 2017 and 2018. Each successive draft appears to have undergone a reduction in scope, if the pagination is reflective of the expanse of the content. Given that drafts need to be purchased at around 100 euros each, this limits the opportunity to review any changes.

That the ISO 14698 two-part standard required an update is not disputed; this author set out the reasons why a revision was much needed back in 2013 (5). The key elements that the author felt should be included were set out in a second article on the necessity for a revised standard for biocontamination control, also issued in 2013 (6). Several of the points made in these articles have not been taken up by the committee.

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What Is Bio-Contamination Control?

Bio-contamination control informs about the state of control of the environment within which an activity happens (7). The environment is controlled, either as a classified cleanroom or a clean facility. Importantly, the environment will inform as to the potential risk of control breakdown, but it does not directly inform as to the risk to the product (8).

Developing well-designed programs is essential to understanding the risks, contamination source, strategies to reduce risks, and for assessing suitable monitoring locations in order to understand whether a hazard is present (9, 10).

DEFINITIONS

Every standard needs a scope and to clearly state its purpose, and EN 17141:2020 does so, indicating that the purpose is with seeking to establish "best practice for establishing and demonstrating control of airborne and surface microbiological contamination in clean controlled environments." In terms of scope, the standard is applicable to pharmaceuticals, biopharmaceuticals, medical devices, other life sciences, healthcare, hospitals, food, and other industries that make use of clean and controlled environments. Given the variation in control expectations between these sectors, whether the amalgamation works is uncertain.

Alert and Action Levels

The standard has a number of definitions, for example, alert and action levels, which are similar to those used by the PDA and which are satisfactory explanations. However, accompanying alert and action levels is reference to a 'target level'. Can one, and more importantly should one, have a target level of contamination? Does this mean normal levels based on a trend assessment of data? The term 'target level' is more customarily associated with statistical control charting (11).

Furthermore, the standard considers limit setting. Here it is stated "the microbiological contamination can be very low and may not conform to normal distributions. In such cases parameters such as average or standard deviations may not be suitable to establish action and alert levels. Instead levels that are likely to be exceeded at defined frequencies should be considered more appropriate for setting action and alert levels."

In this author's experience microbial data rarely ever conforms to normal distribution, and here the standard should be stronger with this regard (12). It also stands that the use of frequencies is not typically acceptable to inspectors as the sole measure of assessing control. With regulators also requiring a numerical level, the standard should describe established approaches like the percentile cut-off approach (which many pharmaceutical microbiologists have been using for several decades).

Other Contradictions with Definitions

Some of the other definitions used as less clear. For example, 'critical zone' is defined as a "designated space within the clean controlled environment used to control microbiological contamination". However, the zone may be critical, but the ability to control it may not be in place or possible. It might be better to discuss the relative risk in relation to a 'critical zone'. A further area where clarity is required with the definition of 'microorganism of interest'. Here this is defined as: "microbiological contamination that has been identified as harmful to the product or the process, or the intended recipient of the product within the clean controlled environment." Here it may be better to use 'specified' organism, as this is more in keeping with the pharmacopeia (and in recognition that the term 'objectionable' is now generally regarded as redundant).

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The definition of 'sterilisation' reflects sometimes varying interpretation of the term. The standard refers to "the validated process used to render a product free from viable microorganisms", yet sterilisation cannot be proven, and it is best understood as a probabilistic concept.

Stepwise Risk Assessment

The standard, in outlining the objectives, presents a particularly good stepwise process for assessing microbial risks. This appears to be based on Hazard Analysis Critical Control Point (HACCP) methodology (a subject excellently covered by Jahnke and Khun) (13). The standard proposes that the microbiological contamination control system should be formed of the following parts (this is not precisely a direct quote, there is some modification made here to make the text easier to follow):

1. Identification of all potential microbiological contamination sources and routes of contamination in the environment. This may also include selected microorganisms of interest.
2. Assessing the risk from these sources and routes and, where appropriate, introducing or improving microbiological contamination control methods to reduce the identified risks.
3. Establishing a monitoring schedule, with valid sampling methods, in order to monitor the microbiological contamination source, or their control methods or both.
4. Establishing alert and action levels with measures to be taken when required, if these levels are exceeded.
5. Verification on a continuing basis, that the microbiological contamination control system is effective and meeting agreed performance parameters by reviewing product contamination rates, environmental monitoring results, risk assessment methods, control methods and monitoring limits and, where appropriate, modifying them accordingly.
6. Establishing and maintaining appropriate documentation; education and training of all staff involved with the clean controlled environment.

Overall, this is a sound approach, and in keeping with established risk assessment principles.

With reference to microbial risks specifically, the standard recommends that the following factors should be considered as part of the risk assessment for what is referred to as a 'clean controlled environment application' (for which we can read pharmaceuticals and healthcare):

- Microbiological species, in relation to survival possibility or associated toxins
- The potential for causing microbiological contamination of the product and/or harm to the intended recipient, such as spoilage of product.
- The product form, which may be dependent upon whether the product contain preservatives, or any potential growth substrates that may prevent growth.
- The intended product target population such as the type of patient.

The list is useful to a point, and the reference to microbial toxins is important (especially as this is often overlooked). The list is based on Sutton's classic schema (albeit unacknowledged), but it is not as well developed. What Sutton recommended, back in 2006, was (14, 15):

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- Total microbial numbers. Although high numbers of non-pathogenic organisms may not pose a health hazard, they may affect product efficacy and/or physical /chemical stability. An unusually high number of organisms seen in the product may also indicate a problem during the manufacturing process, or an issue with a raw material. The high bacterial counts may indicate that the microorganisms are thriving in the product.
- The characteristics of the microorganism, to determine if the organism is a known pathogen. A second characteristic of the microorganism that must be taken into account is the potential for the organism to cause spoilage of the product.
- The use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract).
- Product characteristics. The dosage form is important to consider. Is the product anhydrous or water based? This can have an effect on the ability of microorganisms to proliferate. Does it have sufficient free water to support microbial growth. Is the container designed to minimize contamination and subsequent spoilage?
- The nature of the product: does the product support growth? Does it have adequate antimicrobial preservation?
- The method of application.
- The intended recipient: risk may differ for neonates, infants, the debilitated.
- The use of immunosuppressive agents, corticosteroids.
- The presence of disease, wounds, organ damage.

In terms of the types of microorganisms recovered, the standard offers some sound advice when it comes to fungi, stating that "the presence of moulds and other microbiological contamination, including microorganisms of interest, can be indicators of poor cleaning or poor design and increase the risk of product and/or process contamination." This is a good point and one worth bearing in mind when assessing the facility microbiota.

Sources of Contamination

The standard presents different sources of contamination, which reflected general literature. These are divided into 'prime sources' and 'associated sources'. These have been summarized, from the standard, into a diagram constructed by the author (Figure 1).

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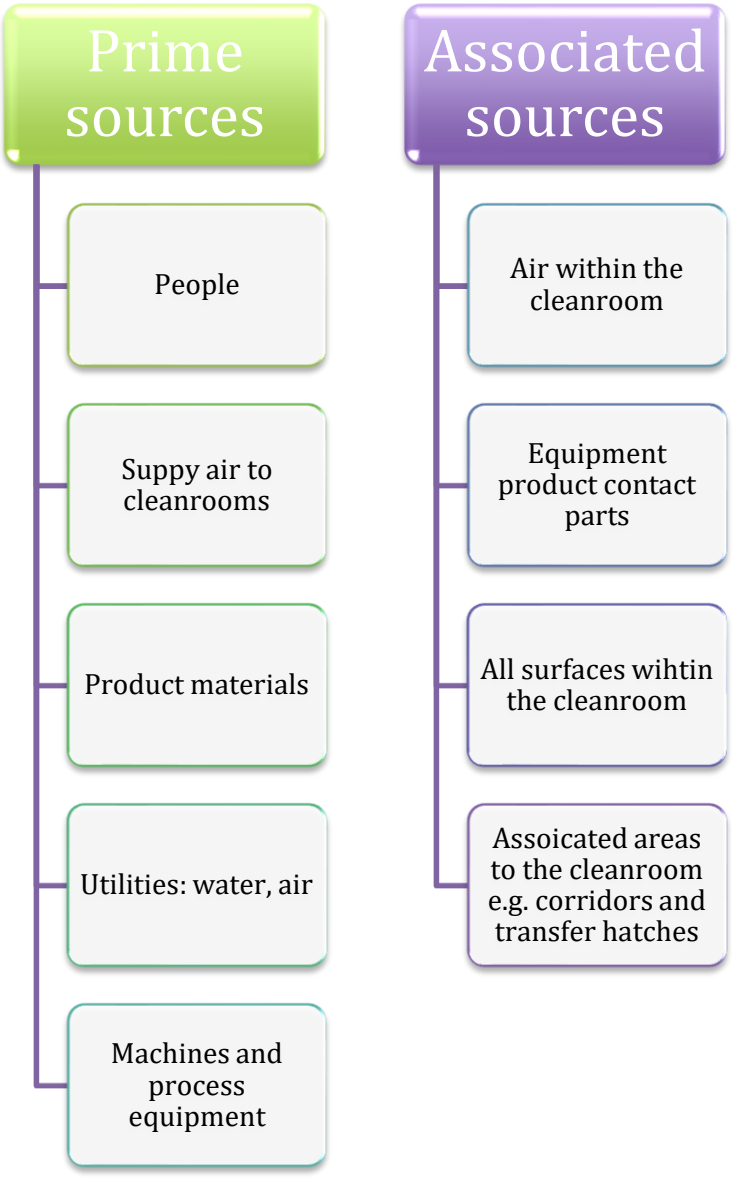

Figure 1: Prime and associated contamination sources

With the factors listed in Figure 1, reference is made to conducting risk assessments. Little information is provided, although there is a cross-reference to other risk assessment standards. In terms of tools, Fault Tree Analysis, Hazard Analysis Critical Control Points, and Failure Modes and Effects Analysis, are each mentioned together with "any other verified system". What constitutes a 'verified system' is not elucidated upon. Some older references are quoted, but little relating to current literature on contamination control or environmental monitoring risk approaches.

The document then proceeds to consider three vectors for contamination: airborne deposition; surface contact; and liquid. At this stage, liquid is said to fall outside the scope of this document. This is a shame, given that water presents a risk in terms of being

both a growth medium for aquatic bacteria and a vector for contamination (from water outlet tubing to puddles on cleanroom floors). Excluding liquids does detract from the scope of the standard.

Environmental Monitoring Plan

Environmental monitoring is an important part of bio-contamination control, once risk assessments have been completed and risk mitigation strategies adopted. The standard infers that environmental monitoring plans are well described (there is actually not that much written) and, as with risk assessment, none of the classic studies are referenced (as have been published in the *Journal of GxP Compliance* or the *Journal of Pharmaceutical Sciences*, as examples).

In terms of the aspects of the plan listed:

1. Locations for monitoring. These are described as being connected to part of a risk assessment, related to the degree of risk. This is a sensible approach. Called out elements for potentially more monitoring are personnel gowning, pass through hatches, and material transfer airlocks. This is based on these activities presenting a potentially higher risk. Also included in the risk considerations is the type of product manufactured.

What is also important to include is open processing, and areas of higher personnel activity within the cleanroom. These are not directly assessed in the standard.

An approach to selecting monitoring locations is expanded under Annex B of the standard for the assessment of clean environments used to manufacture medical devices. This section begins by advising that locations should be selected on the basis of risk; however, the standard then goes on to offer an alternative where the risk approach does not identify any locations of concern. Quite why a risk assessment would fail to identify locations of concern is unclear.

The recommendation in the standard is:

- Active air samples: N/3 with a minimum of one;
- Surface samples: 3 +N/3 on each work area.

Where: N is the minimum number of determined locations according to ISO 14644-1:2015

ISO 14644 is the standard for particle classification of cleanrooms and here sampling locations are selected based on a statistical approach for particles. The approach is based on a statistical method called hypergeometric distribution. This is based on particles not being normally distributed and the statistical method has an in-built confidence interval of 95%. This means when air is sampled at the number of locations specified for particles, there is a 95% level of confidence that 90% of the cleanroom complies (provided the data is satisfactory).

While this has some merit for viable organism in the air, given that most microorganisms in air are attached to other matter and hence could be following a similar distribution, there is no logical basis for applying this for surface samples. The approach may lead to too many samples being taken and a danger that the samples are not actually covering process or equipment flows, or close to the core activity itself.

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For personnel monitoring in relation to medical devices, a generalized assessment is provided (in table B.4). This does not appear to be risk based and there are different ways to look at the matter. Why, for example, is s a sample of the chest from a gown an identified risk site but not the forearm? There is also a curio in relation to taking a 'positive control' of an operator's hairline (presuming he or she has hair) outside of the cleanroom. Why would this be necessary if the media has undergone growth promotion testing? This is presuming the agar plate after incubation can be counted. Moreover, what should the incubation parameters be? The human microbiome project data would suggest that anaerobic bacteria are the predominant organisms residing in this particular niche.

2. The frequency of sampling. Monitoring periods are also related to the degree of risk. There is also reference too frequent sampling that could potentially introduce further risk due to possible intrusion of sampling personnel into a critical zone.

These are useful points, but there is a lack of detail of risk factors to consider (in relation to sterile products manufacturing aligned with Annex A). Moreover, the time of sampling is not addressed (16). As examples, Sandle's schema includes (17):

- Room activity – differences between process, storage, office/administration, washing, sterilising, such as, autoclave operation, sterile filtration and sterile filling.
- Exposure risk: For how long is the product exposed?
- Is open processing involved? If so, for how long?
- Room temperature - Cold, warm or ambient?
- Process stage - Raw material processing, intermediate manufacturing or final formulation?
- Duration of process activities - Short, medium- or long-term relative to all operations
- Water exposure or wet area increases risks.

For medical devices (Annex B), the frequencies of monitoring may be insufficient. The standard at this point reverts to ISO cleanroom classes (rather than EU GMP grades, which is odd for a standard designated 'EN'). For the monitoring frequencies, the ISO class 5 recommendation is correct (for the duration of the activity), but the surrounding environment (which will be ISO class 7, which in operation is equivalent to Grade B) should also be continuous in order to conform with current regulatory expectations.

3. Taking action. With actions to be taken, the standard is contradictory. In one section it states: "The microbiological environmental monitoring plan shall specify the alert conditions under which action is to be taken", which infers alert levels require an action. Later the standard states: "In some cases, alert levels can also be reported, particularly those associated with a multiple or unusual occurrence", which is a better approach, indicating that some alert level patterns may trigger the need for action. However, having two different statements is unhelpful.

4. Trend analysis. The reference to trend analysis is good, and the inclusion of microorganisms, frequency of excursions, and actual data values is well-made. Here, as with other sections, some pictorial examples would be useful (reference is made to Shewhart charts and cumulative sum charts, both of which are useful), which is in keeping with previous literature (18, 19). However, embarking on such an approach requires an assessment of what is normal variation compared with an upward shift.

The importance placed on trending is one of the very good points in the standard. The reasons for this are due to individual results rarely being of significance and a recognition that environmental monitoring techniques can have a degree of variability.

5. The standard shifts from the use of action and alert levels to 'out of specification' (OOS). This is very disappointing. It has taken a number of years for the pharmaceutical microbiology community to steer regulators away from seeing environmental monitoring excursions as 'specifications'. They are not specifications; results should be considered out-of-limits or 'at alert' or 'action' or forming part of an upward trend.

Care should be taken when reacting to individual results for microbiological results are often difficult to interpret. This is for several reasons:

- Microorganisms are ubiquitous in nature and are common environmental contaminants;
- A single result does not by itself indicate a breakdown of environmental control;
- More meaningful data is often assessed from trends (see below);
- The microbiologist has the potential to introduce contaminating microorganisms during sampling and/or testing;
- Microbiological sampling methods are subject to considerable inherent variability.

6. The standard mentions the importance of sample tracking and record keeping. These sections are satisfactory.

7. Integrity of results. The sections on data handling and data record keeping act as useful reminders for laboratories. This connects with previous literature that there are a range of factors that can influence the accuracy of environmental monitoring data (20).

Environmental Monitoring Methods

The environmental monitoring methods described in the standard are the classic culture-based methods, which have been commonplace since the 1960s. With culture media, the standard covers the pre-requisites for media sterility, growth promotion (the standard 50-200% acceptance criteria is set out in the appendix), and the addition of neutralisers where disinfectant residues are a concern. Reference is also made to the assessment of dehydration due to airflow and post-sampling confirmation of growth promotion.

The standard offers some good, general advice on method selection. This involves taking account of:

- The time and duration of the clean controlled environment activities;
- Accessibility into the clean controlled environment for the sampling device;
- The effect of the sampling device on the process or environment to be monitored;
- The efficiency and precision of the sampling method.

The standard proceeds to set out what a manufacturer of an air-sampler should assess. There is also the recommendation that the user periodically assesses volumetric airflow. While these aspects are important, it is noted that under medical devices (in Annex B) there is reference to taking a minimum of 100 litres of air (rather than the standard 1000 litres). The volume recommended is insufficient to provide an accurate assessment of airborne cleanliness, and microbiological samples cannot be extrapolated based on their non-homogenous distribution.

Sections are appended on different models of air-samplers, and it is good to see detail of the d50 value, which is a critical criterion for air-sampler assessment, together with the recommendation of the value being less than 2 µm. Particle size distribution D_{50} is

also known as median diameter or medium value of particle size distribution, it is the value of the particle diameter at 50% in the cumulative distribution. The use of the D_{50} value is one of the important parameters characterizing particle size. That this section is clear and comprehensive is testament to the citing of the important work by Ljungqvist and Reinmüller on this subject (21). The detail on air sampler validation for physical and biological collection efficiency is solid, from the enclose chamber perspective. The recommendation that the use attempt to qualify a new model air-sampler by comparing it to an existing model when sampling in an environment of a relatively high bioburden (such as an EU GMP Grade D equivalent cleanroom) is fraught with complications, not least the likely distribution of organisms in air. Anyone attempting this will no doubt end up taking a very large number of samples in order to achieve any semblance of statistical significance.

With settle plates, a statement is made in relation to the 4 hour exposure time, which runs: "this time may have to be reduced in locations where the media may dry out quickly, e.g. where the plates are exposed to high air flow rates". This may be the case, but the user should attempt to achieve a four exposure first (such as using a larger agar fill or adding glycerine) before electing to shorten the exposure time, especially if there is the requirement to conform to EU GMP Annex 1. For surface samples, more detail on the standardization for taking contact plates in terms of sampling time for contact with the surface, and perhaps pressure (which can be achieved using several commercial applicators) would have made for a useful inclusion. With swabbing, there is no reference to direct plating but instead to plating out the moistening fluid. This assumes that captured microorganisms can be released from the swab tip. With swab tips, literature indicates that flocked swabs are superior to plain swabs for microbial recovery and not referring to this is an omission.

In association with the core methods, reference is made to the use of incubators, which extends to temperature monitoring and temperature mapping. What is set out here is in line with Good Manufacturing Practice (GMP).

Reference is made to rapid methods. The standard indicates that these are non-cultural methods, but this is not always the case. The appendix presents advice on rapid and alternative method qualification, which is a summary of what is already contained within the pharmacopeia. A reference about comparability which goes: "Alert and action levels based on established culture based methods may not be applicable...For example, if the new method has improved sensitivity, results that exceed the alert or action level may occur and may not be indicative of a change in the state of control." This is probably correct, but many regulatory bodies have signalled that limits should not be changed. An improved understanding of the numbers of organisms present should instead drive the cleanroom manager to seek improved controls.

Plate Incubation

In the appendix, there is a description of plate incubation. The standard states: "In general, total count incubation at 30 ^{0}C to 35 ^{0}C for a minimum of 3 days is suitable for the growth of many bacteria and 20 ^{0}C to 25 ^{0}C for a minimum of 4 days is suitable for the growth of some fungi".

With this:

- It is unclear if this is a reference to dual incubation or to single media.
- If it is dual incubation, the order that the incubation is expressed in runs counter to most published studies (including this author's, which recommends a low-to-high temperature regime) (22).
- The incubation times need to be based on a study, and this may vary according to different facilities.

In no cases is any literature to incubation conditions cited. This is perhaps one of the most concerning parts of the standard.

Monitoring Different Environments

The standard has annexes describing the monitoring of different environments. There is insufficient detail here, especially with Annex A (sterile products), and the reader is directed to look at different documents from some (but not all) of the professional associations. Given that these are often contradictory, this does not help. The role of a standard should be to set out what is required and to offer clearer advice. Even where regulations are referred to, where the regulations have gaps these are not covered. For example, EU GMP Annex 1 only covers active air sampling, settle plates and contact plates, yet swabs are needed to assess filling needles and stopper bowls as part of the post-fill assessment of aseptically filled products. The standard should plug this gap and describe swab samples.

The regulations are also applied inconsistently. For example, under medical devices, reference is made to ISO 13408: Aseptic processing of healthcare devices (part 7, which pertains to medical devices), but strangely other parts of this standard are not referenced in relation to sterile pharmaceutical products.

SUMMARY

It is time for a new bio-contamination control standard to help build on regulatory guidance. Almost two decades on from ISO 14698 being issued, EN 17141 arrives. While the standard contains some useful advice, it is unbalanced in its level of detail (lots of information on qualifying volumetric air-samplers, very little on risk assessment. There are also sections of concern, such as the description of incubation conditions that are present as precise descriptors of time, when they cannot be. The standard is also uneven, sometimes contradictory (as with the issue about alert levels); sometimes incorrect (as with the definition about sterility); and sometimes bureaucratic. For instance, with training the reader is directed to ISO 14644 Part 5, last revised in 2004 and not due for review until 2023. Yet ISO 14644-5: 2004 directs the reader back to the two-part ISO 14698 standard, for microbial control, that EN 17141 indicates is defunct.

Hence, the standard does not fully deliver what the pharmaceutical industry, medical device, and healthcare products sector requires (no comment has been offered about the food or hospital sectors). While this article has been admittedly critical in places, hopefully it stimulates discussion and leads to a stronger standard when version 2 appears. This is not a criticism of the work has gone into the creation of the standard, but a criticism of the scope and level of involvement. One reason for the points of concern made, looking at the references, appears to be a lack of involvement of pharmaceutical microbiologists. None of the classic writers are referenced (like Sutton, Cundell, Moldenhauer, Miller or Roesti) (23 – 27). Neither was the global professional body for pharmaceutical microbiology, Pharmig, consulted. Hopefully a redress with occur and the revision process will begin in the short term.

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