

Recommendations for sterilisation tests with Pulsed UV light, on bio-analysis and how to avoid common pitfalls.

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Foreword:

Industries are used to Gamma and E-beam ISO sterilization specs, which are user-universal. The UV (and especially PUV) sterilisation strongly depends on product conditions. For that reason there is no universal rule (and respectively no ISO spec) on how to use PUV- each case requires evaluation tests and piloting, then the FDA (or EFSA) approval. This problem remains for SteriBeam as well, yet our most recent customers agreed to do so for PUV and PEF. The situation with approvals for the PEF sterilization is just the same as for PUV).

A. Sterilization goals

have to match actual production/regulation requirements, and vary from 2 to 6 logs (1 log - 10 times reduction, etc). It requires an analysis of contamination sources.

Quite often this task is neglected by setting "an overkill" sterilization goal of 4 to 6 or even higher log reductions whereas 2 to 3 logs could be sufficient. Besides much higher costs of such sterilization, it is also not so simple to verify especially for packaged products or for products which can shadow parts of UV light. Usual guidelines for sterilization goals are the following:

- A.1. Products manufactured in controlled clean environment with a manual labor usually require an extra or "insurance" sterilization from 2 to 3 logs.
- A.2. Medications and products going into the A-class clean room require 6 logs sterilization.

To estimate sterilization of internal surfaces of packaging or of packaged products, the first step is to measure their [UV transparency in broad UV range \(200 to 400 nm\)](#).

The reason - a flash lamp generates broad and intense UV spectrum in this range, which also can be adjusted to fit a specific sterilization task. A UV transmission spectra allows to find if this or that sterilization task is possible to carry or not. If so, it will allow us to set optimal sterilization parameters and to offer sterilization tests.

SteriBeam offers a service in measuring UV transparency if his local lab does not have a UV spectrophotometer.

B. COMMON BIO-FAILURES DURING UV TESTS:

if a customer wish to buy or to rent our system to try sterilization on its own, its bio personnel has to avoid some common mistakes which will bring negative results.

The major mistake is an attempt to get "fast" results by skipping all statistical and inoculation requirements. Such requirements are set e.g. in the international sterilization spec ISO 11137. This spec was developed for sterilization with electron beams or with Gamma sources and therefore we do not post it on our website so not to overload a customer with reading (it can be found through Google search, if interested).

What is important here is that there is no similar spec for UV sterilization. That is because

UV sterilization is very dependent on properties of a sterilized product which is not the case for e-beam or Gamma sterilization, namely to UV transparency of products, UV shadowing, strong dependence on UV wavelengths and so on). In fact, self-shadowing by bacterial layers of each other (see below) puts a certain limit on UV sterilization in so called "natural multiple contaminations" normally used for inoculation.

However we can use the requirements for statistics and reference samples, accepted at ISO 11137: it uses naturally (or production) acquired microbial load with the test statistic as following:

for 1 log - null occurrence in 10 samples, ,

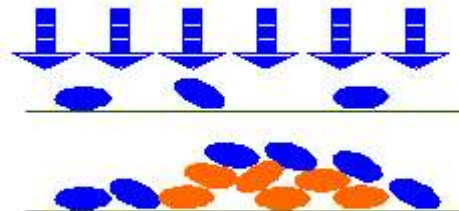
for 2 log - null occurrence in 100 samples,

.....

for 6 log - null occurrence in million samples,

Clearly, that the only way to cut on this high statistic is to make inoculation (sedimentation) of sample products with micro-organisms of interest to a user. Statistic is required to overcome

too few survivors in naturally contaminated samples, (even in reference samples), so inoculation of targeted micro-organisms is a common procedure. Usually it is 2 to 3 logs inoculation and the rest is done with statistic. A higher inoculation is also possible however it has to be done with much of a caution since there is



the MAJOR possible pitfall during a 4 to 6 logs inoculation:

laying multi-layers of micro-organisms where top layers shadow the UV light for underlying micro-organisms: see below:

UV light >

natural contamination >

False (multi-layer) inoculation >

More over, multiple layers of micro-organisms cannot naturally occur on many properly manufactured products (like contact lenses, syringes or vials etc). So to induce such a high inoculation is to follow a false scenario, which results could be mis-leading. Yet mono-layers naturally also do not exist since miro-organisms, once together, form colonies, which are not single layers. It could be a subject of a debate, yet *one has to perform "proportionality tests" and see where it levels out - this will be a limit for a given inoculation:*

- one pulse at a recommended exposure takes 1 or 2 logs out, the second pulse has to get the same reduction, and so has to do the third up to your inoculation goal.

The statistic of tests must be no less than 5 (better 10) samples for each test.

If results are not yet clear, we recommend one more test to be performed with a standard CWUV 254 nm lamp, also on open Petri dishes with the same and half of inoculation.

If tests again do not get you proportionality, then you have to improve your inoculation until you get this proportionality. Only after you get the proportionality, it will be the right time to proceed further with PUV sterilization tests.

SteriBeam also offers to analyse your test protocols at no extra charge once you have purchased or rented our systems.

Please, be confident, that our PUV systems have earned a reputation as high log ultra-fast sterilizers, which is easy to proof once the bio-work is done properly!

C. Recommended micro-organisms, evaluation and protocols:

C.1. Commonly used test micro-organisms:

- Bacillus Subtilis spores (also its sub-class B-thermophiles, or heat resistant spores),
- Bacillus Pumilus spores,
- Aspergillus Niger spores (UV resistant spores),
- Salmonella, E-coli.

C.2 Evaluation method:

is by number of residual-surviving colonies, counted after 2 and then after 7 days following the sterilization. The method has to be sensitive enough to see a difference within one log reduction.

NOTE: do NOT use "colour" strips which equally respond to one and to thousand of survivors - these are used to control a production, not for an evaluation.

C.3 Test protocols:

the goal of test protocols is to control a consistency in tests, and simple verification of results. Protocols have to be for each sample (or product, or its part) and include the date of tests, the name of person who did tests, data on inoculation, data on PUV exposure as read from the system manual (pulse energy and a number of pulses, a position of a sample, see below as Amendment).

Reference samples such as with open Petri dishes, Petri dishes covered with you sample packaging and untreated but inoculated samples have to be logged in their own Protocols, the same as for target sampling.

C.4. Tests statistic.

is the min 10 samples for each case (where each case is a product or its part (if parts of product are differently made), its position to UV light, like external or internal surface of a product to UV, etc.

C.5. Handling samples and the UV unit.

Samples have to be handled with medical rubber gloves by a properly dressed operator. The system and samples should be under a vent hood to avoid an human induced contamination.

All tests have to be done at the lab (or at the facility) where the lab is. Any travel of samples must be fully avoided since a travel bring non-controllable conditions (rise in temperature, in moisture, can bring extra contaminations, etc).

In some cases, when samples are the final product to be vacuum packed and shipped anyway, it is possible to have tests done at SteriBeam and samples set to a lab chosen by a customer. Respective packaging should be with blue-ice and by UPS (or TNT) over-night express with a simple temperature recorder inside.

The desk-top PUV unit and CWUV (UVC or UVB continuous) desk top systems is t be provided by SteriBeam or can be brought and operated by SteriBeam personnel to your site for a fee).

C.6 Bio-labs.

If you have an in-house lab, capable to comply with above conditions, the use it. If not, we can help you to find and to qualify service labs in your area (on your request).

One of world re-known bio-lab chains is www.eurofins.com

Another recognized lab is CCFRA in UK: <http://www.campden.co.uk/>

Amendment. A sample of tests Protocols:

Date: _____

Place _____ Person to do bio-work _____

Tests micro-organisms _____ at the density of _____ Logs

Inoculated as a MONO-LAYER at internal (...) or external (..) surface of a sample

The product sample is taken from the product named _____ coded as _____

The part of the product used in tests: _____

Table of Results.

N/#	1 day check	7 day reduction	number of pulses	pulse J/cm ²
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
Ave:				

Reference Samples results for treated:

N/#	1 day check	7 day reduction	number of pulses	pulse J/cm ²
1				
2				
3				
4				
5				

Reference Samples results for untreated:

The score for 5 samples _____

Signature_____