Guidance on the establishment of timeframes for testing associated with the requirements of ISO 11137, ISO 11737 and ISO 13004.

Foreword:
Discussions in the Microbiology Working Group regarding the timeliness associated with the testing for bioburden, dose establishment, and sterilization dose audits following the manufacture of healthcare products, has led to the following guidance document.

The aim of this document is to assist both manufacturers and laboratories in following the relevant standards and to support their justifications for testing practises in audit situations.

Preface:
It is important to understand that there are two critical factors involved in the successful application of the correct verification dose applied to product when performing Dose Setting / VD_{MAX}^{SD} studies.

a) The performance of accurate bioburden estimations on product in particular those that support microbial growth, and
b) The timeframes involved and the potential for timeframes to influence the accuracy of bioburden estimations and tests of sterility.

The essence of this guidance document assumes the manufacturer has access to a competent microbiological testing facility and competent sterilization personnel, be those “in house” or contracted out. This is a requirement of the relevant standards.

Whilst good practice dictates that bioburden testing, irradiation at the verification dose, and tests of sterility should be performed as soon as practicable, it is also recognised that there are a number of variables outside the manufacturer’s control; transit and delivery times, contract laboratory location and backlog, irradiator timeslots, etc. which can make this difficult to achieve. This list might not be exhaustive.

Therefore, there is a need to have a robust justification in place addressing these factors, to satisfy the requirements of the standards.

This guidance document addresses ways in which manufacturers, sterilization facilities and test houses can mitigate the effects of the timeframes and justify the route chosen to obtain “best practice” whilst maintaining a practical approach.

Introduction:

The ISO 11737, ISO 11137 series and ISO 13004 contain the following statements on the timeliness of testing following the manufacture of a healthcare product;

Bioburden testing: **(See T1 in process flow)**

> *ISO 11737-1, 5.1.3 ‘Consideration shall be given to the timing of the performance of determination of the bioburden relative to manufacturing, because bioburden can change with the passage of time.’*
>
> *See also ISO 11737-1, A 5.1.3. …’ the period of time that elapses between the selection of product samples and the determination of bioburden should be representative of the time period between completion of the last manufacturing step and sterilization of the product.’*

ISO 13004, 5.3.3 "For product capable of supporting microbial growth, the maximum allowable amount of time from manufacture to sterilization of product shall be determined. Storage conditions (including refrigeration of product, if applicable) should be considered as part of this determination. The period of time between the taking of product items from production and the determination of bioburden or the performance of the verification dose experiment should be reflective of the maximum allowable holding time."

"The manufacturing step to use in this determination of the maximum allowable holding time should be the last step before the product would be capable of supporting microbial growth (e.g. the last mixing step for a liquid formulation), and in many cases this might not be the very last manufacturing step prior to sterilization of product"

Verification dose experiment: (during dose establishment or auditing)

ISO 11137-2, 5.3.2. …'the period of time that elapses between the taking of product from production and the performance of the verification dose experiment should reflect the time period between completion of the last manufacturing step and sterilization of product.'

ISO 11737-2, 6.8. … 'The interval of time between exposure of the product to the sterilising agent and performing the tests of sterility on such product shall be as short as practicable'

Bioburden frequency:
Completion of bioburden testing on finished healthcare products that will be radiation sterilized, occurs at a defined frequency as per ISO 11137-1 section 12.1.2 dependent on the method of dose establishment. This can be monthly or every 3 months.

Dose auditing frequency:
A sterility dose audit is required every three months (or up to a maximum of 12 months with documented rationale) as per ISO 11137-1, section 12.1.3 on finished healthcare product that will be radiation sterilized.

Dose establishment frequency:
Dose establishment studies have no defined frequency – they are generally performed for new products, to address changes to product families, or significant changes in bioburden load that can affect dose established, or as a reaction to dose audit failures.

1. The understanding of "lag times" and their criticality:

It is recognised that when performing dose establishment experiments there will be a delay (a 'lag time') between manufacture of product and the application of the verification dose to product as well as between the application of the verification dose and the performance of the test of sterility.

In the case of Method 1 and VD_{max}^{SD} methods (as described in ISO 11137-2 and ISO 13004), bioburden determinations are required as a first step to determine the verification dose to be delivered to samples. The bioburden determinations take time to test, incubate and then analyse results in preparation for the next step of delivering the verification dose, usually to samples from the same batch as that from which the bioburden samples came.

In the case of Method 2 dose establishments, the lag time is even longer. Step one is an incremental dosing series followed by the test of sterility and incubation. In step two the data from step one is evaluated, and an additional radiation dose (verification dose) is applied to products followed by the test of sterility and incubation. Generally, step two is performed on product from one of the same batches

tested in step one. These tests of sterility will take a minimum 14 days of incubation and many days can pass between irradiation dosing prior to the test of sterility taking place. This means that prior to application of the verification dose in step two, the samples have been stored for at least three to four weeks post manufacturing. (The standard does allow for a fourth batch to be used.)

Although this long lag time might seem concerning, a few items should be understood;

1. Method 2 has been in place and performed in this manner for at least four decades. To date there is no data from the industry indicating that this is an unsafe practice.

2. For products that do not support microbial growth; when a dose audit is performed, the verification dose will usually be applied in a much shorter timeframe from manufacturing compared to the timeframe during dose establishment, and there are no indications in the industry that Method 2 dose audits are routinely failing. This means that the extra two or three weeks of time that pass during dose establishment are not creating an inaccurate situation due to a reduction in bioburden count.

3. Although this information is specific to Method 2, the concept also applies to the other methods; the passing of two or three weeks, and the potential reduction in bioburden count, has not been significant enough to cause noticeable problems in the industry.

It is still important for manufacturers to justify the timelines used in routine practise.

NOTE: The requirements for dose establishment, VD_{max}^{SD} experiments, and audit frequencies are clearly defined in ISO 11137, ISO 11737 and ISO 13004.

Since dose establishment and dose audit experiments need to be relevant to the bioburden present at the time of sterilization, consideration must be given to the time period between a) manufacture and testing, and b) manufacture and terminal sterilization. This is what will be considered as the “lag time.”

> *ISO 11737-1, A 5.1.3 states. …’the period of time that elapses between the selection of product samples and the determination of bioburden should be representative of the time period between completion of the last manufacturing step and sterilization of the product’*

Bioburden levels can be subject to change with the passage of time. It is therefore important to determine whether or not the healthcare product can support microbial growth. In such instances it is necessary to understand these changes in bioburden profile over time.

Where the healthcare product does not support microbial growth, the bioburden profile could either remain the same or reduce over time. Generally, it is understood in the industry that if a product is dry (e.g., no water in a liquid state), the product will not support microbial growth. For a dry product which does not support microbial growth, the testing lag time is not generally a concern.

The primary concern for lag times and testing timeframes are those products that do support microbial growth as this will give the greatest opportunity for changes if dosing or testing is not timely.

For example: The microbial load on the product could remain high or even be supported to grow further over time. Following exponential growth there could also be die-off and different microorganisms then surviving at different points over time.

NOTE: When referring to product samples it is important that ‘Product for establishing or auditing the sterilization dose shall be representative of that subjected to routine processing procedures and conditions’ as per ISO 11137-2 section 5.3.1. Products required to be transported to a contract laboratory or irradiator should be packaged appropriately to prevent damage that could affect microbiological characteristics.

1a Product that will support microbial growth:

Products that would be more likely to support or encourage microbial growth can be those with a higher water activity or other liquid / component part that supports growth.

For most health care products (plastic and metal products), visible moisture is required to support microbial growth. With these products high humidity is usually not sufficient to support growth due to the microorganisms needing more than just high humidity to replicate. Thus, the information in this section applies generally to products with liquid water or other liquid / component part that supports growth.

Knowledge of the product and its bioburden profile over time is important to then be able to assess and justify the timing of testing.

The bioburden profile of healthcare products over time can be achieved in one of two ways;

1. Performance of a 'dwell study' where the newly manufactured pre-sterile healthcare product is tested over a defined timeline to assess the changing bioburden levels. (With consideration of the use of more than one batch if some variability between batches is expected or seen.)

2. Gathering of data on a product or product family that has been historically tested at different days since manufacture and building the bioburden profile over time for that product.

A "dwell study" is a process whereby the possible changing bioburden levels are tested at defined time periods, starting as close to Day 0 (date of manufacture) as possible, and continuing through to End Day (typical timeframe between manufacture and terminal sterilization.) Sample numbers should represent those employed for routine bioburden testing. See section 2 for more information.

Note: Samples could also be subject to worst case conditions of temperature/humidity or other relevant condition, to simulate the worst seasonal variation.

Collating the bioburden data in either way will give the manufacturer the information to establish testing timeframes.

Typical approaches that could be used include selecting the testing timeframe where the bioburden levels peak. Alternatively, the timeframe could be selected based on a typical or worst-case lapse of time before product is sterilized.

In circumstances where testing is not carried out in-house, liaison with the contract laboratory is important to establish specified timelines based on the knowledge of the bioburden change over time.

1b Product that does not support microbial growth:

Products that do not support microbial growth (e.g. no water content, no wet nutrient substrates, that results in a hostile environment for microorganism growth), unlike those that support growth, are likely to present highest bioburden levels at point of manufacture, and subsequent delays in testing will result in numbers of microorganisms remaining the same or dropping. Once data is collated to demonstrate that the timeframe for testing does not affect the outcome, it can then be argued that it is not critical for a target day to be set for bioburden testing or verification dose application.

(Note: Products that contain antimicrobial / antifungal properties by design, should be highlighted by the manufacturer to the test house as this property will affect test method design for both bioburden and dose auditing.)

2. Bioburden Profile over time 'Dwell study':

As mentioned above knowledge of the importance of bioburden changes in a product over time can be achieved in different ways;

1. When liquid water is present, performance of a dwell study where the newly manufactured pre-sterile healthcare product is tested over a defined timeline to assess the changing bioburden levels.
2. When liquid water is not present, document as such; no specific timeframe is required to be established.
3. Gathering of data on a product or product family that has been historically tested at different days since manufacture and building the bioburden profile over time for that product.

Dwell study example:

An example is for healthcare product to be tested at Day 0 (day of manufacture), and subsequent days or weeks following. Days selected for testing should be representative of what might be expected as far as the time for terminal sterilization for example; 0,1,2,3,4,5,6,7,14,21,28,35. Sample numbers chosen should be similar to the sample numbers employed for bioburden testing under normal, routine testing conditions. (Test samples / timeframes should be tailored to represent routine processing procedures)

Table 1: Example of dwell testing data for microbial growth supporting product:

Day	Bioburden value average CFU/product Example 1
0	100
1	200
2	500
3	1100
4	2000
5	3000
6	3000
7	2000
Week 2	1100
Week 3	500
Week 4	300
Week 5**	200

**Duration of the dwell study or data review should extend to the reasonable worst-case scenario of sterilization of the healthcare product taking into account shipment times and scheduling at the irradiation supplier. This example stops at week 5 but in practise testing might be needed beyond this period to reflect larger timescales between manufacture and sterilization.

In general, from dwell testing data collection the manufacturer can then provide a robust justification for their approach. These approaches could include for example:

- due to the product not supporting microbial growth, no significant change is expected in the bioburden levels over time and therefore there is no specific target day between manufacture of product and testing of bioburden or sterility dose audit, OR,

- due to the product being capable of supporting microbial growth, a significant change in the bioburden levels or types can be expected and has been demonstrated over the timeline tested and therefore a target day is set (the worst-case, highest bioburden day, or the day by which it is determined product has to be sterilized) for the bioburden/application of verification dose to be performed.

- Microbial characterisation can be required

As per ISO 11737-1, A 4.4.1 ‘’Bioburden test results do not generally fit a mathematical distribution model. Therefore, consideration of measurement uncertainty, precision and bias may not be necessary, except for evaluating the overall competency of the laboratory. For bioburden test methods, the measurement of uncertainty, precision and bias are taken into account by the determination of the bioburden recovery efficiency.’’

Bioburden data do not follow a standard distribution model therefore natural variation can account for differences in the results during a dwell study – not necessarily enhanced growth or die-off of the microorganisms. The selection of the quantity of samples at each timepoint can ensure the influence of this variation is lessened.

The obligation lies with the manufacturer to understand the timelines and agree with the contract sterilizer/laboratory what they require as a bioburden test and irradiation of verification dose timeline.

The manufacturer should communicate with the contract lab / irradiator that their testing timeline is based on their product bioburden profile knowledge and that the specified testing timelines are being achieved.

3. Time between irradiation with verification dose and the performance of the test of sterility:

It is widely expected that the test of sterility has to occur as soon as is reasonably practical from the time of delivery of the verification dose. As per ISO 11737-2, *6.8 … ‘The interval of time between exposure of the product to the sterilising agent and performing the tests of sterility on such product shall be as short as practicable.’*

The timeliness of the application of the verification dose is important. The timeliness of the performance of the sterility test following the irradiation of verification dose is also important. The primary concern is that microorganisms can be damaged but not inactivated by the verification dose, but then might become inactivated as additional time passes.

A ‘false positive’ in a test of sterility actually presents more of a manufacturer’s risk whereby they then have to investigate and react accordingly.

A ‘false negative’ in a test of sterility presents a more direct patient risk as this can result in product being confirmed as sterile when in fact it is not.

Following dosing, it should be ensured that the laboratory carries out testing as soon as possible as per ISO 11737-2, 6.8 note above. It can be necessary to consider the storage of samples prior to testing in order to preserve the microbial state.

Test labs and manufacturers should coordinate and take practical steps to schedule the dosing and testing when resource and incubation space is available in order to achieve the required timescales and not cause delay between dosing and testing.

The timely transit of samples between dosing site and testing location should also be planned to minimise die off / stress of surviving microorganisms.

4. Time between manufacture and irradiation at a verification dose for dose establishment:

(See T2 in process flow)

As discussed in the introduction it is recognised that when conducting a dose establishment there is usually a delay between manufacture of healthcare products and the application of the verification dose to product.

Specific examples:

1. A Method 1 or VD_{max}^{SD} dose establishment for multiple production batches requires bioburden testing of 3 lots of healthcare product – incubation of up to 7 days before a calculation can be made to select the verification dose. This then means the verification dose cannot be applied until after this incubation and calculation step. This inevitably means time has passed since the manufacture of healthcare product and the verification dose being applied.

2. A Method 2 dose establishment requires incremental dosing be performed followed by 14 days incubation and only when this is complete can a calculation of the verification dose be made. This then means the confirmatory verification dose experiment test cannot be performed until at least 14 days following the incremental dosing step.

The question often asked is, has the bioburden changed significantly in this time that could affect the dose establishment result and can the affect of this change be mitigated?

Manufacturers can employ one of the following techniques here:

1. The preferred option and best practise would be to use data obtained from dwell time study to demonstrate that product bioburden remains stable over the passage of time between receipt of samples and dosing,

   OR where data is not available, cannot be collated or does not support, justify the use of option 2 or 3;

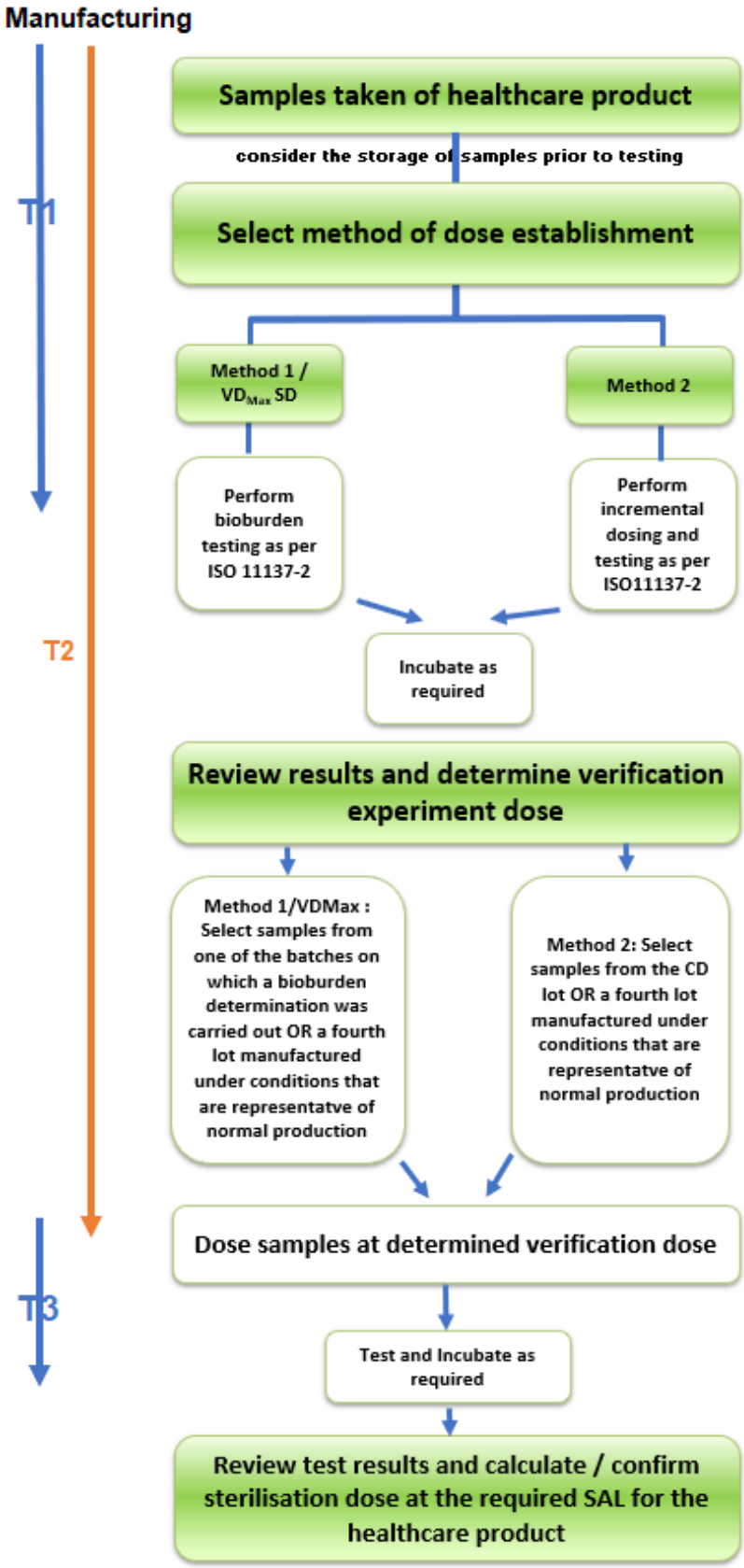
2. If the product supports microbial growth consider holding the test samples in the fridge or other suitable storage condition during the first stages of the dose establishment to ensure any change in bioburden is minimised.

3. Where practicable take samples from a fourth batch manufactured under conditions that are representative of normal production to perform the verification dose experiment if the product bioburden is expected to change significantly in the 2+ week time period.

The key aspect here is to know the healthcare product bioburden profile well and a dwell study such as that described above, can provide the justification to support that there is no significant change over time and therefore no impact as the healthcare product waits to be dosed (if indeed the testing has shown this).

See the following process flow for the approaches that can be taken to dose establishment sampling and testing timeframes.

Process flow for dose establishment approaches



5. Dealing with excursions to required timeframes:

This section applies to product for which it has been deemed necessary to establish a required timeframe.

Once in routine processing if there is a failure to achieve the required timeframe for bioburden testing / dosing of dose audit samples or even terminal sterilization of finished product – there can be actions the manufacturer takes to mitigate the associated affects on sterility;

1. The manufacturer can run the dwell study testing above to a longer timespan to ensure even the most extreme worst-case scenario is covered.

2. The manufacturer could retain pre-sterile samples from batches made in order that extra bioburden testing could be conducted should the timeframe of terminal sterilization be exceeded. That way they could assess the bioburden level at the point of sterilization and therefore understand the effect on the success of the terminal sterilization of the product. In this situation the resulting bioburden types after the exceeded timeframe are as important as the bioburden numbers.
   NOTE: (Consideration should be given on how these samples are stored and should reflect actual conditions experienced by the product prior to sterilization.)

3. If the dose audit testing was outside of the desired timeframe, evaluate the data obtained and compare to the data obtained when within the timeframe, to ascertain if there is any difference.

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