

## **Pharmacopeial sterility test: The statistical limitations of sampling**

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### Pharmacopeial sterility test: The statistical limitations of sampling

For aseptically filled products, as well as for many terminally filled products, the sterility test is a mandatory product release test. It is, however, statistically poor at detecting anything other than gross contamination (this limitation has been addressed in a number of studies (1)). This limitation relates to the few numbers of articles tested (2). For batches in excess of 500 filled containers, the pharmacopeia only require that twenty samples are included in the sterility test set. This sample size appears to have been set arbitrarily, and it does not provide a statistically significant population with which to estimate sterility (3). Although it is unclear how this sample size was derived, the number is grounded, in part, through the sterility test being a destructive test (each article tested via the sterility test is not available for the patient) and therefore to maximise the availability of the batch by using as few units as possible.

It remains, nonetheless, that the sample size of 20 provides no confidence that the sterility of a batch of pharmaceutical items has not been compromised.

In relation to sampling, limitations not only apply to the low number of samples tested but also to the difficulties in selecting a sample representative of all significant events during batch filling (4). This is important because contamination is unlikely to be uniformly distributed throughout the batch and thus random sampling cannot detect contamination with absolute certainty. This is of particular importance with aseptic filling where batch specific events can occur. It is possible that certain events can be captured, such as interventions into the aseptic core, where the vials exposed at the time of the activity can be incorporated into the sterility test set (notwithstanding that all events cannot be captured in this way).

The difficulty in detecting a low level of contamination from a batch of filled product can be illustrated by way of an example. To illustrate this an equation is required. Consider that  $p$  refers to the proportion of contaminated containers in a batch, and  $q$  the proportion of corresponding non-contaminated containers. Arranging these, we have two possible expressions:

$$p + q = 1$$

or,

$$q = 1 - p$$

#### **Example 1: selecting specific contaminated containers from a batch**

To take an example, where we assume that we have a specific batch where 10% of the containers are contaminated. From this batch, two items are withdrawn

On this basis, the probability of a single item taken at random showing contamination can be given by the following expression:

$$p = 0.1 \text{ [i.e., } 10\% = 0.1]$$

Whereas the probability of such an item being non-contaminated is can be represented by the following expression:

$$q = 1 - p = 1 - 0.1 = 0.9$$

The probability status of the two contaminated articles being detected may be expressed in three different forms. These are:

(a) When both items are contaminated :  $p^2 = 0.01$

(b) When both items are non-contaminated :  $q^2 = 0.81$

(c) When both items are non-contaminated :  $q^2 = (1 - p)^2 = (0.9)^2 = 0.81$ , and

(d) When one item is contaminated and the other one is non-contaminated :  $1 - (p^2 + q^2)$

$$\text{or} = 1 - (0.01 + 0.81) = 1 - (0.82)$$

or = 0.18 i.e., =  $2pq$

### Example 2: Chance of passing a sterility test where contamination is present in a batch

This premise can be expanded further. To take a particular sterility test, with a sample size of  $n$  containers, the ensuing probability  $p$  of duly accomplishing  $n$  consecutive pass results is represented by the following expression:

$$qn = (1 - p)n$$

To look at this another way, in mathematic terms, if  $n$  is the number of containers tested, and  $p$  is the proportion of contaminated containers, and  $q$  is the proportion of non-contaminated containers, then:

$$p + q = 1$$

From this it follows that:

$$\text{The probability of rejection} = 1 - (1 - p)n$$

For example, the pharmacopoeia test for sterility passes batches from which 20 items have been tested and shown not to be non-sterile. If the proportion of non-sterile items in a batch were to be one in one hundred the expression above would be solvable as follows:

$$(1-p) = 0.99$$

Take the logarithm of 0.99

$$\text{Log}_{10} 0.99 = -0.0044$$

Solve for  $(1-p)n$  by multiplication

$$(1-p)n = (-0.0044) \times 20 = (-0.088)$$

Withdraw from logarithmic form by taking the anti-log

$$\text{Anti-log of } (-0.088) = 0.817$$

Therefore we can show mathematically that with an incidence of one non-sterile item in one hundred, there is greater than 80% chance of passing the Test for Sterility.

A follow-on concern, based on the uncertainty of detecting contamination, is that if a batch was to fail the sterility test (due to low level contamination) and the batch was then subject to a repeat sterility test (which is a controversial area discussed later in this book), there is a reasonable chance that it would pass on the second occasion.

In mathematical terms, the probability of passing a batch with some contaminated containers on a retest is:

$$(1 - p) [2 - (1 - p)n]$$

For example, if there is a sample size of 10 and the contamination rate is 5%, then if the sterility test is repeated with another sample size of 10 units the probability of including a contaminated item is again 0.4. However, the probability of both tests being positive reduces as it becomes  $0.4 \times 0.4 = 0.16$  which is lower than the probability of the first test, and hence in 84 of 100 such tests the batch will be accepted as sterile.

**Example 3: Further cases examining the difficulty of detecting non-sterility in a batch**

To illustrate this concept further, the ensuing values for various levels of  $p$  (proportion of contaminated containers present in a batch) having essentially a constant sample size are as provided in the table below.

The table values are probabilities (in this, the probability of contamination). Probabilities are given a value between 0 (0% chance or will not happen) and 1 (100% chance or will happen) (Feller, 1968). Therefore, a probability of 0.4 means that on four out of every 10 occasions it is likely that an event will occur (in this context, if a sterility test was conducted 10 times, on 4 occasions the test would detect contamination and on 6 occasions it would not detect contamination). Hence, the sterility test fails to detect low levels of contamination and it is often unknown where in a batch of product the contaminating articles are located.

If different sample sizes are taken (based upon  $(1 - p)n$  factor), the table also illustrates that the sample size enhances the probability of detection (interestingly the Australian Therapeutics Goods Agency required a sample size of 30 items in its Test for Sterility until the 1990's. This was the only regulatory agency ever to set a sample size above 20 units). However, the sensitive of detection only becomes meaningful where a relatively high proportion of the items are contamination ( $\geq 5\%$ ) and where relatively large sample sizes are taken ( $\geq 50$ ). To take another example, with a sample size of 10 and a contamination rate of 5% the probability of including a contaminated item is 0.4.

In reality, where contamination does occur this is normally tied to specific events during batch filling and this would be, in all probability, less than 0.1% of the batch. With the recommended sterility test size required by the pharmacopeia of 20 items, then the relative insensitivity of the sterility test is all too apparent (5).

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