Where warranted, a risk-based assessment of the relevant factors is conducted by personnel with specialised training in microbiology and the interpretation of microbiological data. For raw materials, the assessment takes account of processing to which the product is subjected, the current technology of testing and the availability of materials of the desired quality.

Table 5.1.4.-2. – *Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use*

	TAMC (CFU/g or CFU/ml)	TYMC (CFU/g or CFU/ml)
Substances for pharmaceutical use	10^3	10^2

Appendix: Special Ph. Eur. provision for herbal medicinal products consisting solely of one or more herbal drugs (whole, reduced or powdered): quantificative test for *E. coli*

Use the following protocol.

Sample preparation and pre-incubation. Prepare a sample using a 10-fold dilution of not less than 1 g of the product to be examined as described in general chapter *2.6.12*, and use the quantities corresponding respectively to 0.1 g, 0.01 g and 0.001 g (or 0.1 ml, 0.01 ml and 0.001 ml) to inoculate a suitable amount (determined as described under 3-4 of general chapter *2.6.13*) of casein soya bean digest broth, mix and incubate at 30-35 °C for 18-24 h.

Selection and subculture. Shake the container, transfer 1 ml of casein soya bean digest broth to 100 ml of MacConkey broth and incubate at 42-44 °C for 24-48 h. Subculture on a plate of MacConkey agar at 30-35 °C for 18-72 h.

Interpretation. Growth of colonies indicates the possible presence of *E. coli*. This is confirmed by identification tests.

Note the smallest quantity of the product that gives a positive result and the largest quantity that gives a negative result.

Determine from the following table the probable number of bacteria.

Results for each quantity of product			Probable number of bacteria per gram or millilitre of product
0.1 g or 0.1 ml	0.01 g or 0.01 ml	0.001 g or 0.001 ml	
+	+	+	> 10^3
+	+	-	< 10^3 and > 10^2
+	-	-	< 10^2 and > 10
-	-	-	< 10

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5.1.5. APPLICATION OF THE F_0 CONCEPT TO STEAM STERILISATION OF AQUEOUS PREPARATIONS

The following chapter is published for information.

The F_0 value of a saturated steam sterilisation process is the lethality expressed in terms of the equivalent time in minutes at a temperature of 121 °C delivered by the process to the product in its final container with reference to micro-organisms possessing a theoretical *Z*-value of 10.

The total F_0 of a process takes account of the heating up and cooling down phases of the cycle and can be calculated by integration of lethal rates with respect to time at discrete temperature intervals.

When a steam sterilisation cycle is chosen on the basis of the F_0 concept, great care must be taken to ensure that an adequate assurance of sterility is consistently achieved. In addition to validating the process, it may also be necessary to perform continuous, rigorous microbiological monitoring during routine production to demonstrate that the microbiological parameters are within the established tolerances so as to give an SAL of 10^{-6} or better.

In connection with sterilisation by steam, the *Z*-value relates the heat resistance of a micro-organism to changes in temperature. The *Z*-value is the change in temperature required to alter the *D*-value by a factor of 10.

The *D*-value (or decimal reduction value) is the value of a parameter of sterilisation (duration or absorbed dose) required to reduce the number of viable organisms to 10 per cent of the original number. It is only of significance under precisely defined experimental conditions.

The following mathematical relationships apply:

$$F_0 = D_{121}\,(\log N_0 - \log N) = D_{121} \log IF$$

D_{121} = *D*-value of the reference spores (*5.1.2*) at 121 °C;

N_0 = initial number of viable micro-organisms;

N = final number of viable micro-organisms;

IF = inactivation factor.

$$Z = \frac{T_2 - T_1}{\log D_1 - \log D_2}$$

D_1 = *D*-value of the micro-organism at temperature T_1;

D_2 = *D*-value of the micro-organism at temperature T_2.

$$IF = \frac{N_0}{N} = 10^{t/D}$$

t = exposure time;

D = *D*-value of micro-organism in the exposure conditions.

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5.1.9. GUIDELINES FOR USING THE TEST FOR STERILITY

The purpose of the test for sterility (*2.6.1*), as that of all pharmacopoeial tests, is to provide an independent control analyst with the means of verifying that a particular material meets the requirements of the European Pharmacopoeia. A manufacturer is neither obliged to carry out such tests nor precluded from using modifications of, or alternatives to, the stated method, provided he is satisfied that, if tested by the official method, the material in question would comply with the requirements of the European Pharmacopoeia.

PRECAUTIONS AGAINST MICROBIAL CONTAMINATION

Aseptic conditions for performance of the test can be achieved using, for example, a class A laminar-air-flow cabinet located within a class B clean room, or an isolator

GUIDANCE TO MANUFACTURERS

The level of assurance provided by a satisfactory result of a test for sterility (the absence of contaminated units in the sample) as applied to the quality of the batch is a function of the homogeneity of the batch, the conditions of manufacture and the efficiency of the adopted sampling plan. Hence for the purpose of this text a batch is defined as a homogeneous collection of sealed containers prepared in such a manner that the risk of contamination is the same for each of the units contained therein.

In the case of terminally sterilised products, physical proofs, biologically based and automatically documented, showing correct treatment throughout the batch during sterilisation are of greater assurance than the sterility test. The circumstances in which parametric release may be considered appropriate are described under *5.1.1. Methods of preparation of sterile products*. The method of media-fill runs may be used to evaluate the process of aseptic production. Apart from that, the sterility test is the only analytical method available for products prepared under aseptic conditions and furthermore it is, in all cases, the only analytical method available to the authorities who have to examine a specimen of a product for sterility.

The probability of detecting micro-organisms by the test for sterility increases with their number present in the sample tested and varies according to the readiness of growth of micro-organism present. The probability of detecting very low levels of contamination even when it is homogenous throughout the batch is very low. The interpretation of the results of the test for sterility rests on the assumption that the contents of every container in the batch, had they been tested, would have given the same result. Since it is manifest that every container cannot be tested, an appropriate sampling plan should be adopted. In the case of aseptic production, it is recommended to include samples filled at the beginning and at the end of the batch and after significant intervention.

OBSERVATION AND INTERPRETATION OF RESULTS

Conventional microbiological/biochemical techniques are generally satisfactory for identification of micro-organisms recovered from a sterility test. However, if a manufacturer wishes to use condition (d) as the sole criterion for invalidating a sterility test, it may be necessary to employ sensitive typing techniques to demonstrate that a micro-organism isolated from the product test is identical to a micro-organism isolated from the test materials and/or the testing environment. While routine microbiological/biochemical identification techniques can demonstrate that 2 isolates are not identical, these methods may not be sufficiently sensitive or reliable enough to provide unequivocal evidence that 2 isolates are from the same source. More sensitive tests, for example molecular typing with RNA/DNA homology, may be necessary to determine that micro-organisms are clonally related and have a common origin.