Table 5.1.3.-1. - *Parenteral and ophthalmic preparations*

		Log reduction				
		6 h	**24 h**	**7 d**	**14 d**	**28 d**
Bacteria	A	2	3	-	-	NR*
	B	-	1	3	-	NI**
Fungi	A	-	-	2	-	NI
	B	-	-	-	1	NI

*NR: no recover

**NI: no increase

The A criteria express the recommended efficacy to be achieved. In justified cases where the A criteria cannot be attained, for example for reasons of an increased risk of adverse reactions, the B criteria must be satisfied.

Table 5.1.3.-2. - *Topical preparations*

		Log reduction			
		2 d	**7 d**	**14 d**	**28 d**
Bacteria	A	2	3	-	NI
	B	-	-	3	NI
Fungi	A	-	-	2	NI
	B	-	-	1	NI

The A criteria express the recommended efficacy to be achieved. In justified cases where the A criteria cannot be attained, for example for reasons of an increased risk of adverse reactions, the B criteria must be satisfied.

Table 5.1.3.-3. - *Oral preparations*

	Log reduction	
	14 d	**28 d**
Bacteria	3	NI
Fungi	1	NI

The above criteria express the recommended efficacy to be achieved.

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5.1.4. MICROBIOLOGICAL QUALITY OF PHARMACEUTICAL PREPARATIONS

The following chapter is published for information.

This general chapter presents 2 sets of recommended acceptance criteria for microbiological quality. The 1st set of criteria corresponds to the 1st sets of methods in general chapters 2.6.12 and 2.6.13. In the same way that the 1st sets of tests in chapters 2.6.12 and 2.6.13 are to be replaced in future by the 2nd sets, so the 1st set of criteria in this chapter will be replaced by the 2nd set. Where authorised, the 2nd set of criteria may be used instead of the 1st set before replacement of the latter. The 2nd set presents criteria developed in co-operation with the Japanese Pharmacopoeia and the United States Pharmacopeia to achieve harmonised requirements.

A. METHOD OF THE EUROPEAN PHARMACOPOEIA

In the manufacture, packaging, storage and distribution of pharmaceutical preparations, suitable measures must be taken to ensure their microbiological quality. The pharmaceutical preparations should comply with the criteria given below.

Category 1

Preparations required to be sterile by the relevant monograph on the dosage form and other preparations labelled sterile.

– Test for sterility (*2.6.1*).

Category 2

Preparations for topical use and for use in the respiratory tract, except where required to be sterile, and transdermal patches.

– Total viable aerobic count (*2.6.12*). Not more than 10^2 micro-organisms (aerobic bacteria plus fungi) per gram, per millilitre or per patch (including the adhesive and backing layer).

– Transdermal patches: absence of enterobacteria and certain other gram-negative bacteria, determined on 1 patch (including the adhesive and backing layer). Other preparations: not more than 10^1 enterobacteria and certain other gram-negative bacteria per gram or per millilitre (*2.6.13*).

– Absence of *Pseudomonas aeruginosa*, determined on 1 g, 1 ml or 1 patch (including the adhesive and backing layer) (*2.6.13*).

– Absence of *Staphylococcus aureus*, determined on 1 g, 1 ml or 1 patch (including the adhesive and backing layer) (*2.6.13*).

Category 3

A. *Preparations for oral and rectal administration.*

– Total viable aerobic count (*2.6.12*). Not more than 10^3 bacteria and not more than 10^2 fungi per gram or per millilitre.

– Absence of *Escherichia coli* (1 g or 1 ml) (*2.6.13*).

B. *Preparations for oral administration containing raw materials of natural (animal, vegetable or mineral) origin for which antimicrobial pretreatment is not feasible and for which the competent authority accepts microbial contamination of the raw material exceeding 10^3 viable micro-organisms per gram or per millilitre. Herbal medicinal products described in category 4 are excluded.*

– Total viable aerobic count (*2.6.12*). Not more than 10^4 bacteria and not more than 10^2 fungi per gram or per millilitre.

– Not more than 10^2 enterobacteria and certain other gram-negative bacteria per gram or per millilitre (*2.6.13*).

– Absence of *Salmonella* (10 g or 10 ml) (*2.6.13*).

– Absence of *Escherichia coli* (1 g or 1 ml) (*2.6.13*).

– Absence of *Staphylococcus aureus* (1 g or 1 ml) (*2.6.13*).

Category 4

Herbal medicinal products consisting solely of one or more herbal drugs (whole, reduced or powdered).

A. *Herbal medicinal products to which boiling water is added before use.*

– Total viable aerobic count (*2.6.12*). Not more than 10^7 bacteria and not more than 10^5 fungi per gram or per millilitre.

– Not more than 10^2 *Escherichia coli* per gram or per millilitre (see Appendix).

B. *Herbal medicinal products to which boiling water is not added before use.*

- Total viable aerobic count (*2.6.12*). Not more than 10^5 bacteria and not more than 10^4 fungi per gram or per millilitre.
- Not more than 10^3 enterobacteria and certain other gram-negative bacteria per gram or per millilitre (*2.6.13*).
- Absence of *Escherichia coli* (1 g or 1 ml) (*2.6.13*).
- Absence of *Salmonella* (10 g or 10 ml) (*2.6.13*).

B. HARMONISED METHOD: MICROBIOLOGICAL QUALITY OF NON-STERILE PHARMACEUTICAL PREPARATIONS AND SUBSTANCES FOR PHARMACEUTICAL USE

The presence of certain micro-organisms in non-sterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has a potential to adversely affect the health of the patient. Manufacturers therefore have to ensure a low bioburden of finished dosage forms by implementing current guidelines on Good Manufacturing Practice during the manufacture, storage and distribution of pharmaceutical preparations.

Microbial examination of non-sterile products is performed according to the methods given in general chapters *2.6.12* and *2.6.13 (B. Harmonised method)*. Acceptance criteria for non-sterile pharmaceutical products based upon the total aerobic microbial count (TAMC) and the total combined yeasts/moulds count (TYMC) are given in Tables 5.1.4.-1 and 5.1.4.-2. Acceptance criteria are based on individual results or on the average of replicate counts when replicate counts are performed (e.g. direct plating methods).

When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:

- 10^1 CFU: maximum acceptable count = 20;
- 10^2 CFU: maximum acceptable count = 200;
- 10^3 CFU: maximum acceptable count = 2000, and so forth.

Table 5.1.4.-1 includes a list of specified micro-organisms for which acceptance criteria are set. The list is not necessarily exhaustive and for a given preparation it may be necessary to test for other micro-organisms depending on the nature of the starting materials and the manufacturing process.

If it has been shown that none of the prescribed tests will allow valid enumeration of micro-organisms at the level prescribed, a validated method with a limit of detection as close as possible to the indicated acceptance criterion is used.

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

Table 5.1.4.-1. – *Acceptance criteria for microbiological quality of non-sterile dosage forms*

Route of administration	TAMC (CFU/g or CFU/ml)	TYMC (CFU/g or CFU/ml)	Specified micro-organisms
Non-aqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli* (1 g or 1 ml)
Aqueous preparations for oral use	10^2	10^1	Absence of *Escherichia coli* (1 g or 1 ml)
Rectal use	10^3	10^2	-
Oromucosal use Gingival use Cutaneous use Nasal use Auricular use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml)
Vaginal use	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Candida albicans* (1 g or 1 ml)
Transdermal patches (limits for one patch including adhesive layer and backing)	10^2	10^1	Absence of *Staphylococcus aureus* (1 patch) Absence of *Pseudomonas aeruginosa* (1 patch)
Inhalation use (special requirements apply to liquid preparations for nebulisation)	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 ml) Absence of *Pseudomonas aeruginosa* (1 g or 1 ml) Absence of bile-tolerant gram-negative bacteria (1 g or 1 ml)
Special Ph. Eur. provision for oral dosage forms containing raw materials of natural (animal, vegetal or mineral) origin for which antimicrobial pretreatment is not feasible and for which the competent authority accepts TAMC of the raw material exceeding 10^3 CFU per gram or per millilitre	10^4	10^2	Not more than 10^2 CFU of bile-tolerant gram-negative bacteria (1 g or 1 ml) Absence of *Salmonella* (10 g or 10 ml) Absence of *Escherichia coli* (1 g or 1 ml) Absence of *Staphylococcus aureus* (1 g or 1 ml)
Special Ph. Eur. provision for herbal medicinal products consisting solely of one or more herbal drugs (whole, reduced or powdered):			
– herbal medicinal products to which boiling water is added before use	10^7	10^5	Not more than 10^2 CFU of *Escherichia coli* (see Appendix) (1 g or 1 ml)
– herbal medicinal products to which boiling water is not added before use	10^5	10^4	Not more than 10^3 CFU of bile-tolerant gram-negative bacteria (1 g or 1 ml) Absence of *Escherichia coli* (1 g or 1 ml) Absence of *Salmonella* (10 g or 10 ml)

In addition to the micro-organisms listed in Table 5.1.4.-1, the significance of other micro-organisms recovered is evaluated in terms of:

- use of the product: hazard varies according to the route of administration (eye, nose, respiratory tract);
- nature of the product: its ability to support growth, the presence of adequate antimicrobial preservation;
- method of application;
- intended recipient: risk may differ for neonates, infants, the debilitated;
- use of immunosuppressive agents, corticosteroids;
- presence of disease, wounds, organ damage.

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.

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* (under section B, Harmonised method), 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*, section B, Harmonised method) 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 *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.