the obtained slope and to test the conformity of the estimated intercept (value = 0) versus the obtained intercept. For example, if the estimated slope is b with a standard deviation $s_{(b)}$ of 0.090 with 5 concentrations of micro-organisms, calculate $t = (b - 1)/s_{(b)}$. For intercept a, with standard deviation equal to $s_{(a)}$, $t = (a - 0)/s_{(a)}$. Compare these values to the Student's t at 5 per cent, for 13 degrees of freedom (3 tests, 5 concentrations). Acceptance criterion: if the t values obtained are less than the Student's t, the method is exact in the applied range. In the case that there is no conformity for the slope (slope different from 1) or for the intercept (intercept different from 0) the method is not exact over the applied range.

Qualitative or semi-quantitative evaluations. Use the alternative procedure described for setting the limit of detection. Calculate the proportion of false negatives for bioluminescence and for the pharmacopoeial method over all tested dilutions. Compare the extent of false negatives for the 2 or 3 concentrations of micro-organisms just under the detection limit (for example 5 CFU/inoculum, 2.5 CFU/inoculum or 1.25 CFU/inoculum) giving a positive result. By definition, the detection limit corresponds to 0 per cent of false negatives. Acceptance criterion: the percentage of false negatives for the bioluminescence method at sample concentrations below the detection limit must be equal to or lower than that of the pharmacopoeial method.

Range

This is the interval between the lowest and the highest concentrations of micro-organisms where linearity, precision and accuracy have been demonstrated.

Robustness

The information is given by the supplier.

VALIDATION FOR THE ACTUAL INTENDED USE

In the example given, there was no need to determine the accuracy and detection limit in the presence of the product. The validation consists of 3 parts, verifying:

- *phase 1*: the fertility of the medium in the presence of the product;
- *phase 2*: the absence of interference from the product that may increase or inhibit ATP production;
- *phase 3*: the testing of the product in parallel with the pharmacopoeial method.

These 3 parts of validation are performed on 3 independent tests using for example at least 2 different batches of product.

Phase 1: fertility of the medium in the presence of the product

If the product has a known high contamination level (more than 500 micro-organisms per gram or millilitre) the incubation step is unnecessary, the micro-organisms can be detected directly. In this case testing the fertility of the medium in the presence of the product is not necessary. However, pharmaceutical products are generally contaminated at a much lower level and growth of the micro-organism is necessary to obtain detection with bioluminescence. It must therefore be proven that the product does not inhibit the growth of micro-organisms under the conditions of the test. In order to do so, separately add inoculum at not more than 100 CFU for each test micro-organism into the portion of medium containing the product. For bioluminescence in tube or microtitre plate, perform the bioluminescence test. For bioluminescence on membrane, incubate at 30-35 °C or 20-25 °C for 5 days and count the bioluminescent colonies on the membrane. Acceptance criterion: the test is positive (bioluminescence in tube or microtitre plate); the quantitative recovery of the micro-organism is at least 70 per cent (bioluminescence on membrane).

Phase 2: search for interference of the product

The objective is to show that the product does not add stray light or non-microbial ATP (does not lead to false positive result: criterion A) or does not decrease the ATP detection (does not lead to a false negative result: criterion B).

Bioluminescence in tube or microtitre plate

A. Perform the bioluminescence test with the culture broth alone and with the culture broth in the presence of the product. Determine the RLU value for culture broth alone and the RLU value for culture broth in the presence of product.
B. Perform the bioluminescence test with the culture broth alone and the culture broth in the presence of ATP. Determine the response coefficient for ATP concentration in per cent.

Acceptance criterion:

- *criterion A*: the RLU value of culture broth in the presence of product is less than twice the RLU value of culture broth alone (if criterion A is not satisfied, it is necessary to determine a specific threshold for this product);
- *criterion B*: the RLU value of culture broth in the presence of product and ATP is within the interval 25 per cent to 200 per cent of the RLU value of culture broth in the presence of ATP.

Bioluminescence on membrane: perform the complete bioluminescence test to search for interference. Acceptance criterion: the recovery of micro-organisms is greater than or equal to 70 per cent and not more than 200 per cent.

Phase 3: analysis of the product in parallel with the pharmacopoeial method

Perform the test according to the validated method for the product concerned in parallel with the pharmacopoeial method to show the relationship between the 2 methods for the product concerned, on 3 independent tests and using at least 2 different batches. Express the result as positive or negative in a certain quantity (bioluminescence in tube or microtitre plate) or express the count per filtered quantity (bioluminescence on membrane). Acceptance criterion: results must be correlated with the pharmacopoeial method.

01/2008:50107

5.1.7. VIRAL SAFETY

This chapter provides general requirements concerning the viral safety of medicinal products whose manufacture has involved the use of materials of human or animal origin. Since viral safety is a complex issue, it is important that a risk assessment is carried out. Requirements to be applied to a specific medicinal product are decided by the competent authority.

Where the risk of viral contamination exists, complementary measures are used as appropriate to assure the viral safety of medicinal products, based on:

- selection of source materials and testing for viral contaminants;
- testing the capacity of the production process to remove and/or inactivate viruses;
- testing for viral contamination at appropriate stages of production.

Where appropriate, one or more validated procedures for removal or inactivation of viruses are applied.

Further detailed recommendations on viral safety, including validation studies, are provided, in particular, by the *Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses (CPMP/BWP/268/95)* of the Committee for Proprietary Medicinal Products, and the *ICH guideline Q5A: Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin* (including any subsequent revisions of these documents).

Requirements concerning immunological products for veterinary use are dealt with in the monographs *Vaccines for veterinary use (0062)* and *Immunosera for veterinary use (0030)* and related general chapters.

Risk assessment

A risk assessment with respect to viral safety is carried out where materials of human or animal origin are used as ingredients of medicinal products or in the manufacture of active substances, excipients or medicinal products.

The principle of the risk assessment is to consider various factors that may influence the potential level of infectious particles in the medicinal product and factors related to the use of the medicinal product that determine or influence the viral risk to the recipients.

The risk assessment takes into consideration relevant factors, for example:

- the species of origin;
- the organ, tissue, fluid of origin;
- the potential contaminants in view of the origin of the raw material and the history of the donor(s), preferably including epidemiological data;
- the potential contaminants from the manufacturing process (for example, from risk materials used during manufacture);
- the infectivity and pathogenicity of the potential contaminants for the intended recipients of the medicinal product, taking account of the route of administration of the medicinal product;
- the amount of material used to produce a dose of medicinal product;
- controls carried out on the donor(s), on the raw material, during production and on the final product;
- the manufacturing process of the product and its capacity to remove and/or inactivate viruses.

The risk assessment can be based mainly on the manufacturing conditions if these include rigorous inactivation steps (for example, for gelatin etc., and products terminally sterilised by steam or dry heat as described in the general texts on sterility (*5.1*)).

04/2010:50108

5.1.8. MICROBIOLOGICAL QUALITY OF HERBAL MEDICINAL PRODUCTS FOR ORAL USE

This chapter presents recommended acceptance criteria for microbiological quality of herbal medicinal products.

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 the potential to adversely affect the health of the patient. Manufacturers have therefore 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*, *2.6.13* and *2.6.31*. 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 below.

Acceptance criteria are based on individual results or on the average of replicate counts when replicate counts are performed (e.g. direct plating methods).

A list of specified micro-organisms for which acceptance criteria are set can be found below. 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.

A. Herbal medicinal products containing herbal drugs, with or without excipients, intended for the preparation of infusions and decoctions using boiling water (for example herbal teas, with or without added flavourings)

TAMC (*2.6.12*)	Acceptance criterion: 10^7 CFU/g Maximum acceptable count: 50 000 000 CFU/g
TYMC (*2.6.12*)	Acceptance criterion: 10^5 CFU/g Maximum acceptable count: 500 000 CFU/g
Escherichia coli (*2.6.31*)	Acceptance criterion: 10^3 CFU/g
Salmonella (*2.6.31*)	Absence (25 g)

B. Herbal medicinal products containing, for example, extracts and/or herbal drugs, with or without excipients, where the method of processing (for example, extraction) or, where appropriate, in the case of herbal drugs, of pre-treatment reduces the levels of organisms to below those stated for this category

TAMC (*2.6.12*)	Acceptance criterion: 10^4 CFU/g or CFU/mL Maximum acceptable count: 50 000 CFU/g or CFU/mL
TYMC (*2.6.12*)	Acceptance criterion: 10^2 CFU/g or CFU/mL Maximum acceptable count: 500 CFU/g or CFU/mL
Bile-tolerant gram-negative bacteria (*2.6.31*)	Acceptance criterion: 10^2 CFU/g or CFU/mL
Escherichia coli (*2.6.31*)	Absence (1 g or 1 mL)
Salmonella (*2.6.31*)	Absence (25 g or 25 mL)

C. Herbal medicinal products containing, for example, extracts and/or herbal drugs, with or without excipients, where it can be demonstrated that the method of processing (for example, extraction with low strength ethanol or water that is not boiling or low temperature concentration) or, in the case of herbal drugs, of pre-treatment, would not reduce the level of organisms sufficiently to reach the criteria required under B

TAMC (*2.6.12*)	Acceptance criterion: 10^5 CFU/g or CFU/mL Maximum acceptable count: 500 000 CFU/g or CFU/mL
TYMC (*2.6.12*)	Acceptance criterion: 10^4 CFU/g or CFU/mL Maximum acceptable count: 50 000 CFU/g or CFU/mL
Bile-tolerant gram-negative bacteria (*2.6.31*)	Acceptance criterion: 10^4 CFU/g or CFU/mL
Escherichia coli (*2.6.31*)	Absence (1 g or 1 mL)
Salmonella (*2.6.31*)	Absence (25 g or 25 mL)

It is recognised that for some herbal medicinal products the criteria given above under A, B or C for TAMC, TYMC and bile-tolerant gram-negative bacteria cannot be met because of the typical level of microbial contamination. Higher acceptance criteria may be applied on the basis of a risk assessment that takes account of qualitative and quantitative characterisation of the bioburden and the intended use of the medicinal product.

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.

01/2009:50109

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