Microbial Examination of Non-sterile Products

Tepnel Pharma Services

For a variety of reasons, there are a number of problems that can be associated with the microbiological contamination of pharmaceutical preparations and their ingredients. Therefore, each manufacturer is expected to develop microbial specifications and testing protocols for their pharmaceutical products such that patient safety is ensured throughout the manufacturing process (manufacture, packaging, storage and distribution).

In this article we will consider the microbial examination of non-sterile products.

Microbial Contamination and the Absence of Specific Pathogens

In the manufacture of pharmaceutical preparations, the majority of non-sterile products have testing methods derived from pharmacopoeial monographs which require manufacturing practices and controls which limit microbiological presence/absence in accordance with the appropriate microbiological purity criteria, included in the monographs.

In terms of the European Pharmacopoeia, the microbial contamination of non-sterile products is evaluated using the methods given in the general chapters, 2.6.12 and 2.6.13[1].

The significance of microorganisms in non-sterile pharmaceutical products has to be evaluated and tested with consideration of the intended delivery mechanism, the intended use of the product, the nature of the product, and the potential hazard to the patient should something go wrong. The presence of certain microorganisms in non-sterile preparations may also have the potential to reduce or even inactivate the therapeutic activity of the product, which has a potential to adversely affect the health of the patient.

Microbial Contamination

To maintain the safety of any pharmaceutical formulation it is critical that manufacturers understand and routinely monitor their processes, products and personnel to prevent microbial contamination. For non-sterile products, it is recognised and accepted that a bioburden may exists but it equally important that the bioburden is controlled to be within acceptable limits.

Chapter 2.6.12 of the European pharmacopoeia provides guidance on test methods that facilitate the quantitative evaluation of micro-organisms under aerobic conditions. A number of different methods are presented but it is the membrane filtration method and the plate count methods (pour plate and surface spread) that are most commonly used. In both cases a portion of the sample is added to an appropriate medium to ascertain the quantitative number of micro-organisms in the sample. The results are obtained as the number of colony forming units (CFU's) per gram or millilitre of sample tested. It is important to understand that this is not the actual number of micro-organisms present in the sample but rather a count of those organisms that are most likely to grow under favourable growth conditions.

To ensure that these growth conditions are optimal the effectiveness and validity of the method to be used is ascertained by using bacterial test strains at a known concentration of approximately 100 colony forming units per ml. There are five defined test strains which cover the spectrum of bacterial, yeast and fungal contaminants and in each case the count of the recovered test organisms cannot be more than a factor of five away from the calculated value of the inoculum[2].

In all circumstances the suitability of the counting method needs to consider the presence of the product and the characteristics of the product itself. The following table provides guidance on the best approach to sample preparation however it is recognised that where none of the following work, then an alternative approach should be developed.

Formulation	Limits
Water soluble products	Dissolve or dilute 1 part in 10 parts in buffered sodium chloride – peptone solution (pH 7.0), phosphate buffer (pH 7.2) or casein soya bean digest.
Non fatty products, insoluble in water	Suspend 1 part in 10 parts of buffered sodium chloride – peptone solution (pH 7.0), phosphate buffer (pH 7.2) or casein soya bean digest adding a surface active agent to aid suspension if necessary.
Fatty products	Dissolve in isopropyl mystrate or mix with either sterile polysorbate 80 or another non-inhibitory sterile surface active agent making a 1 part in 10 parts dilution
Fluids or solids in aerosol form	Using aseptic transfer techniques, transfer either directly onto a membrane filter or into a sterile container. Note – use either the total contents or a defined number of metered doses from each of the containers to be tested.
Transdermal patches	Place the patches "sticky side up" on either a sterile glass or plastic tray, cover with a sterile porous material and then transfer aseptically to a suitable volume of the chosen diluent containing in activators and shake vigorously for 30 minutes.

It is important that recovery of not more than 100 CFU/ml of control organisms is demonstrated, but note that the volume of suspension added should never exceed 1% of the volume of diluted product.

The total aerobic microbial count (TMAC) is considered to be equal to the number of CFU found using casein soya bean agar. If any colonies of fungal growth are recovered they should be counted as a part of the TMAC. The total

combined yeasts/moulds (TYMC) is considered to be equal to the number of CFU found using sabouraud-dextrose agar, note any colonies of bacteria are detected on this agar should be considered a part of the TYMC. Where an acceptance criteria is prescribed, then it should be interpreted as follows, 10^1 CFU is equal to a maximum acceptable count of not more than 20, 10^2 CFU us equal to a maximum acceptable count of not more than 200, and so on.

Absence of Specific Pathogens

The significance of specific microorganisms in non-sterile pharmaceutical products needs be evaluated in terms of the preparation, the intended use of the product, the nature of the product, and the potential hazard to the user. The presence of certain microorganisms in non-sterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product, which has a potential to adversely affect the health of the patient.

Section 5.1.4 of the European Pharmacopoeia provides a table of acceptance criteria for microbiological quality of non-sterile dosage forms (see below). Whilst the list should not be considered to be exhaustive, it does present a baseline position from which, depending on the preparation, the results of testing can be assessed (relative to formulation type and patient risk) and depending on the nature of the starting materials and the mechanism of manufacture, additional micro-organisms can be added.[3]

The selection of the specific pathogens listed in the below table is because they serve as indicators of potential contamination. Their detection in any formulation above acceptable limits indicates the potential to cause infection, should there be inadequate controls and checks deployed during manufacturing and downstream activity.

Route of Administration	TAMC (CFU/g or CFU/ml)	TYMC (CFU/g or CFU/ml)	Specified Micro-organism
Non-aqueous preparation for oral use	10^3	10^2	Absence of E. coli in 1g or 1ml
Aqueous preparation for oral use	10^2	10^1	Absence of E. coli in 1g or 1ml
Rectal use	10^3	10^2	-
Oromucosal use Gingival use Cutaneous use Nasal use Auricular use	10^2	10^1	Absence of S. aureus in 1g or 1ml Absence of P. aeruginosa in 1g or 1ml
Vaginal use	10^2	10^1	Absence of S. aureus in 1g or 1ml Absence of P. aeruginosa in 1g or 1ml Absence of C. albicans in 1g or 1ml
Transdermal patches	10^2	10^1	Absence of S. aureus in 1 patch Absence of P. aeruginosa in 1 patch
Inhalation use (special requirements apply to liquid preparations for nebulisation)	10^4	10^1	Absence of S. aureus in 1g or 1ml Absence of P. aeruginosa in 1g or 1ml Absence of bile-tolerant-gram-negative bacteria in 1g or 1ml
Special Ph. Eur provision for oral dosage forms containing raw materials of natural (animal, vegetal or mineral) origin for which antimicrobial pre-treatment is not feasible and for which the competent authority accepts TAMC of the raw material exceeding 10^3 CFU/g or CFU/ml	10^2	10^2	Not more than 10^2 CFU of bile-tolerant-gram-negative bacteria in 1g or 1ml Absence of Salmonella in 10g or 10mls Absence of E. coli in 1g or 1ml Absence of S. aureus in 1g or 1ml

The following table provides more detail on why the specific pathogens were selected and their importance as markers of cGMP compliance and control.

Pathogen	Potential
Escherichia Coli	Most E. coli strains are harmless and commonly found in the lower intestine of warm blooded organisms, but some serotypes are pathogenic and can cause serious infections.
Staphylococcus aureus	S. aureus is a member of the normal flora of the body, frequently found in the nose, respiratory tract on the skin and the lower reproductive track of women. Although S. aureus is not always pathogenic it is a common cause of skin infections. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins that bind and inactivate antibodies.
Pseudomonas aeruginosa	P. aeruginosa is found in soil, water, skin flora and most man-made environments around the world. It can cause disease in plants and animals including humans. It is a multi-drug resistant organism, recognised for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses. The organism is considered opportunistic insofar as serious infection often occurs as a consequence of existing diseases or conditions – most notably cystic fibrosis and traumatic burns
Candida albicans	C. albicans is an opportunistic pathogenic yeast that is a common member of the human gut flora. It does not proliferate outside the human body. It is detected in the gastrointestinal tract and mouth in 40-60% of healthy adults. It is usually a commensal organism but can become pathogenic in immunocompromised individuals under a variety of conditions.
Bile Tolerant gram –ve bacteria	Bile-tolerant-gram-negative bacteria belong to the gut flora, gut microbiota or gastrointestinal microbiota, which is the complex community of microorganisms that live in the digestive tracts of humans and other animals, including insects. Their presence indicates potential contamination of a potential animal origin.

Anti-microbial activity and neutralisation

It is important to recognise and mitigate against any inherent anti-microbial activity of both the product and formulation. Neutralising agents may therefore be added to any selective media with the express intent of inactivating any anti-microbial activity that may mask the result of the test. A typical neutralising fluid can be composed of simple salts along with various concentrations of polysorbate 80 and lecithin, sterilised at 121°C for 15 mins. Types of anti-microbial agents that need to be considered include, phenolics, organo-mercurials, halogens and quaternary ammonium compounds.

Conclusion

The Absence of Specific Pathogens test is used to demonstrate that pharmaceutical formulations are produced under controlled conditions and that patient safety is not compromised. The reason for the selection of the specific pathogens is to give an indication of the probable sources of contamination. As such monitoring for the Absence of Specific Pathogens should be performed on a regular basis.

Tepnel Pharma Services' in-house Microbiology Laboratory routinely tests for Absence of Specific Pathogens and has over 30 years of experience with more than 1000 validated methods across a wide variety of formulations.

For all your microbiological testing requirements and further information on Tepnel Pharma Services please contact:

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2. European Pharmacopoeia (Ph. Eur) 9th Edition, section 2..12, Microbiological examination of Non-sterile products: Total viable aerobic count, EDQM Council of Europe.
3. European Pharmacopoeia (Ph. Eur.) 9th Edition, section 5.1.4, Microbial Quality of Pharmaceutical Preparations, EDQM Council of Europe.