



# Microbial Limits Testing: risk assessments for objectionable organisms

**Microbial Limits Testing (MLT) is used to determine whether a non-sterile pharmaceutical product complies with an established specification for microbial quality. This is an important series of tests designed to limit the likelihood of any adverse incident occurring as a result of a high level of microorganisms being present in a non-sterile product. By setting limits on the numbers of bacteria present in the product under test you inherently reduce the likelihood of this occurring.**

The harmonised test methods and specifications used to ensure the microbial quality of pharmaceuticals are described in the British Pharmacopoeia (BP), European Pharmacopoeia (Ph Eur), Japanese Pharmacopoeia (JP) and US Pharmacopoeia (USP).

The Microbial Limit Testing described in these four pharmacopoeias is performed in two parts and is designed to perform the qualitative and quantitative determinations of specific viable microorganisms present in pharmaceutical products.

These parts consist of the Microbial Enumeration Test which is a quantitative enumeration of mesophilic bacteria and fungi (yeasts, moulds) that grow under aerobic conditions and the Tests for Specified Microorganisms which test for the presence of *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Clostridium* species, and Bile-tolerant Gram-negative bacteria.

An essential part of this Pharmacopoeial testing is identifying any objectionable organisms that may be present in the sample. The USP chapter <1111> and Ph Eur section 5.1.4 state that:

*“The significance of microorganisms in non-sterile pharmaceutical products should be evaluated in terms of the use of the product, the nature of the product, and the potential hazard to the user.”*

Therefore, while the Tests for Specified Microorganisms indicate requirements to test for certain organisms depending on the product type, these lists are not exhaustive, and for certain sample preparations it may be necessary to test for other microorganisms. This is dependent on the nature of the product, its starting materials and the manufacturing process.

There is also now the expectation that the significance of other microorganisms recovered from the Microbial Limit Tests should be evaluated to see if they are objectionable to the product. The responsibility for determining whether a microorganism is objectionable belongs to the manufacturers and they must use a risk assessment based approach of the relevant factors to confirm this.

The FDA also addresses the need for non-sterile products to be free of objectionable organisms as set forth in 21 CFR 211.113 and 21 CFR 211.165. The factors that the USP, Ph Eur and FDA state for consideration when performing a risk based assessment into objectionable organisms are summarised below:

- The characteristics of the microorganism
- The number of microorganisms present
- The nature of the product as in whether or not the product supports growth
- Whether the product has adequate antimicrobial preservation
- The use of the product, due to the fact that hazard varies according to the route of administration via the eye, nose, or respiratory tract
- The method of application
- The presence of disease, wounds, or organ damage
- The intended recipient as risk may differ for neonates, infants, and the elderly
- The use of immunosuppressive agents and corticosteroids.

It is recommended that these risk based assessments are conducted by personnel with specialised training in microbiology and the interpretation of microbiological data.

Using the information provided from the USP and Ph Eur, one of the first factors that a manufacturer should consider is whether the organism is a known pathogen or opportunistic pathogen. A good place to start researching this is the FDA's 'Bad Bug Book', as it provides more microorganisms which are capable of causing disease than those listed in the Pharmacopoeias.

It is also important to know the number of organisms present, especially when considering the infective dose and virulence of the organism. If a high number of non-pathogenic organisms are seen it may not pose a health risk, but their presence may affect the product stability and efficacy.

The product interference of the organism with active ingredients, test methods, product stability or container closure system may need to be assessed. If interference is observed, then the organism is determined to be objectionable to the product. High microbial counts may indicate that the microorganisms are thriving in the product, therefore it is important to know if the product supports microbial growth.

Another factor to consider when assessing if the product supports microbial growth, is the pH. If the pH of the product is in the same range for ideal growth of organisms, then this could cause microbial growth.

Whether the product is anhydrous or water based can also have an effect on the ability of microorganisms to proliferate as, if there is sufficient water activity in the product, then this may support microbial growth. The USP chapter <112> provides some representative examples of the water activity required to support the growth of different microorganisms. It is generally accepted that a water activity of 0.91 is required to support bacterial growth and a level of 0.7 or higher is needed to support fungal growth. Knowing the water activity of organisms of concern can then allow an assessment of the relative risk.

It is also worth considering if there is adequate antimicrobial preservation and whether the product contains antimicrobials, noting that antimicrobials are often only effective against some and not all organisms. If microorganisms proliferate then there is also a risk that the Total Aerobic Microbial Count and Total Yeast and Mould Count limits may be breached.

The use of the product and method of application or dosage must also be examined. For example, consider whether the product is a topical, oral or nasal preparation. The importance of identifying all recovered isolates from Microbial Limits Testing will depend upon the product and its intended use. If the product is an oral dosage form, then some bacteria may be tolerated in the product if they are not bile-tolerant due to the conditions in the stomach. In some cases, it may be acceptable to only identify isolates from the Microbial Enumeration Tests when testing is showing high levels. However, for other products such as inhalants and nasal solutions, there is a high concern of microbiological contamination therefore all recovered isolates should be identified. If the organism is known to infect via the route of the product administration, then it can be deemed as objectionable.

Finally, it should be considered in the risk assessment the intended recipient of the product and whether the product will be used for neonates, infants, the elderly or the immunocompromised. All of these target populations are more susceptible to infection than healthy individuals, therefore there may be a high risk of infection from an opportunistic pathogen.

It is important for product developers and manufacturers to remember that these tests are part of general safety testing of the product and should be given appropriate consideration and attention. Failure to assess for the presence of both specified and objectionable organisms can lead to lengthy regulatory delays, costly product recalls and potentially even harm to patients.

Given these potential issues, it is vital to the product development life cycle that Microbial Limits Testing is performed correctly by qualified technicians, as it can provide a good early indication of issues in manufacturing, formulation and packaging of pharmaceutical products before they reach the market.

#### References

- USP Chapter <111> Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use
- EP Chapter 5.1.4 Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use
- Code of Federal Regulations, Title 21, Food and Drugs (Government Printing Office, Washington, DC), Part 211.113(a).
- Code of Federal Regulations, Title 21, Food and Drugs (Government Printing Office, Washington, DC), Part 211.165(b).
- USP Chapter <112>, Application of Water Activity Determination To Nonsterile Pharmaceutical Products.
- Sandle T. The Importance of Water Activity for Risk Assessing Pharmaceutical Products. *J Pharm Microbiol.* 2016, 2:1.
- Sutton, S. 2006. How to Determine if an Organism is "Objectionable". *PMF Newsletter* July, 2006.
- Sutton, S. 2006. Microbial Limits Tests; The Difference Between "Absence of Objectionable Microorganisms" and "Absence of Specified Microorganisms". *PMF Newsletter* June, 2006.



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