

# A regulatory perspective: Examples from microbiological quality control of non-sterile medicinal products

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## Introduction

The Croatian OMCL acts as a part of the national authority of the Republic of Croatia, Agency for Medicinal Products and Medical Devices (HALMED). Our key activity, regulated by the Medicinal Products Act (Official Gazette, 76/13, 90/14, 100/18), is post-marketing surveillance of the quality of medicinal products. The microbiological attributes of a medicinal product are an indispensable part of every medicinal product quality specification. For non-sterile medicinal products, the first requirement is to minimize the level of microbial contamination, i.e. to lower the total number of microorganisms present below an acceptable limit. Additionally, the emphasis of non-sterile product microbiological quality is avoiding at all costs the presence of specified (objectionable) microorganisms. The European Pharmacopeia (Ph.Eur) 10.8. (07/2022), chapter 5.1.4., defines both requirements with acceptance criteria, depending on the nature and route of administration of the product. Product compliance with the requirements for microbiological quality is of critical importance for ensuring patient safety. In this short review, we present results from routine microbiological quality control of non-sterile medicinal products, and some of the contamination findings, for the period from 2017 to 2022. Additionally, we present results from a mini marketing surveillance of microbiological quality control of selected probiotics from the market, namely of the only two probiotics registered as a medicinal product, as listed in Medicinal Products Database, HALMED [Internet], 2022. The remaining probiotics examined in this surveillance are under the Croatian national law regulated as a food supplement, and are not included in this review.

## Materials and methods

### Materials

*Samples:* The samples of medicinal products were sampled from the market, from various wholesalers. The examined samples were considered as representative, and the tested quantity was in line with the provisions of European Pharmacopeia (Ph.Eur.) 10.7. (04/2022). Total number of medicinal products tested in the period from January 2017 to January 2022, is 1074. Samples are grouped according to Ph.Eur. acceptance criteria: 987 for oral use (non-aqueous and aqueous), 69 for oromucosal, gingival, cutaneous, nasal or auricular use, 4 for vaginal use, 12 for rectal use, 1 transdermal patch and 1 medicinal product with special requirements that apply to liquid preparations for nebulization. Probiotics were sampled from a local pharmacy. Total number of samples was 2, both for oral use.

*Media:* all of the media used for testing is Ph.Eur. compliant media, commercially available from bioMerieux. Growth promotion is done on every batch/new shipment of media.

*Reference strains:* all of the strains used for testing are Ph.Eur. compliant strains, commercially available from Microbiologics: ATCC 6538, ATCC 9027, ATCC 8739, ATCC 14028, ATCC 10231, ATCC 6633, ATCC 16404 (all EZ-CFU One Step).

### Methods

The methods used for examination of medicinal products are pharmacopeial methods described in chapters 2.6.12. (*Microbial examination of non-sterile products: Microbial Enumeration Test*) and 2.6.13. (*Microbial examination of non-sterile products: Test for Specified Microorganisms*) of the European Pharmacopeia (Ph.Eur.)

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10.8. (07/2022). Suitability of the method for each product is either performed by the manufacturer (data in Marketing Authorization) or performed by the OMCL. The method used for examination of probiotic contaminants is a pharmacopeial method described in chapter 2.6.36. (*Microbial enumeration of live biotherapeutic products: Test for Enumeration of Microbial Contaminants*) of the European Pharmacopeia (Ph.Eur.), 10.8. (07/2022). Identification of growth is done following Gram staining, by using Vitek 2 Compact and bioMerieux identification cards.

## Results and discussion

All of the samples examined during the given period complied with the specification requirement for microbiological quality, i.e. we observed **no out-of-specification results**. However, we present examples of bioburden that was under the acceptable limits, with identified microorganisms.

### *Non-aqueous and aqueous medicinal products for oral use*

*Example 1.* A medicinal product with the active substance *fosfomycin trometamol*, dosage form granules for the preparation of oral solution, was examined by membrane filtration method, dilution 1:10. Growth was observed on TSA (*Trypase Soy agar*) plates and the result (mean for 2 plates) for TAMC (*Total Aerobic Microbial Count*) was 27 CFU/g (limit  $\leq 10^3$  CFU/g). Identified microorganisms were: *Aerococcus viridans* (94%, GP), *Alloiococcus otitis* (93%, GP), *Staphylococcus lentus* (95%, GP), *Kocuria rosea* (98%, GP).

*Example 2.* A medicinal product with the active substance *pioglitazone hydrochloride*, dosage form tablets, was examined by direct inoculation method (surface spread), 1:100 dilution. Growth was observed on TSA plates and the result for TAMC was 693 CFU/g (limit  $\leq 10^3$  CFU/g). The identified microorganism was *Acinetobacter lwoffii* (99%, GN).

### *Transdermal patches*

*Example 3.* A medicinal product with the active substance *fentanyl*, was examined by direct inoculation method (surface spread), dilution 1:10. Growth was observed on TSA plates and the result for TAMC was 18 CFU/patch (limit  $\leq 10^2$  CFU/patch). The identified microorganism was *Staphylococcus hominis ssp hominis* (96%, GP). All of the microorganisms identified as contamination in medicinal products in routine control are bacteria, mainly GP cocci, a part of normal human

microbiota or environmental flora and infrequently pathogens in humans. There were no yeasts and moulds detected.

### *Probiotics*

Medicinal products with the active substance *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium animalis subsp. lactis* (BB-12), dosage form hard capsule, and with the active substance *Bifidobacterium animalis subsp. lactis* (BB-12), dosage form powder for preparation of oral suspension, were examined by direct inoculation (pour plate) method.

In both cases, observed growth of contaminating microorganisms was below the limits set by Ph.Eur. Identified microorganisms were *Aerococcus viridans* (87%, GP) and *Kocuria kristinae* (85%, GP), both frequent environmental and human microbiota microorganisms.

## Conclusion

The Ph.Eur. provides clear requirements for microbiological quality of non-sterile medicinal products. Nevertheless, as discussed in Sandle (2016), following good practice it is up to the regulatory body, as well as the manufacturer, to perform identification and risk assessment of all microorganisms present in the medicinal product, even if the number of colonies is acceptable and species are not listed as objectionable in Ph.Eur.

As shown in Sandle (2016), the bioburden of a final product is a direct consequence of control measures implemented by the manufacturer under GMP requirements. From the pool of various samples of medicinal products examined in HALMED's OMCL for microbiological quality in the period of 2017.-2022., and lack of *out-of-specification* results, we can conclude that the measures implemented by manufacturers are efficient in assuring the microbiological quality and thus safety of medicinal products on the market.

## References

- Council of Europe. European Pharmacopeia (Ph.Eur.) 10.8. (07/2022); Available at: [European Pharmacopoeia Online \(edqm.eu\)](https://www.edqm.eu)
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