

Benefits and Limitations of Species Identification

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■ Abstract

Microbial contaminants can have a huge impact on product safety. Thus, microbiological testing is required for many products and production processes. Good production hygiene is of the utmost importance for contamination control of pharmaceutical manufacturing processes for sterile and non-sterile drugs. In Chapter 5.1.4 the European Pharmacopeia suggests microbiological limits for final products. However, for environmental monitoring there are no detailed requirements for specific organisms. The question arises, to what level an isolate from the manufacturing environment should be identified. How beneficial is species identification? Is it always necessary to have a name for an isolated contaminant in production processes?

This article discusses the need for identification of microbial contaminants in pharmaceutical production environments, especially in the light of the information required to establish an appropriate disinfection regime. In the context of contamination control and production hygiene measures, the need for unconditional identification using high-tech methods is reflected.

■ Keywords

GMP | Production Hygiene | Validation | Identification | Contaminants

Safety of Products

Pharmaceutical drugs need to be safe, i.e. they must not pose an infection risk for the patients. In production processes effective contamination control strategies (CCS) are key, and microbiological testing is performed at multiple stages. For products meant to be sterile, the absence of any microorganism is a non-negotiable requirement. For non-sterile products the situation is by far not as clear.

To ensure safety of products, European Pharmacopeia (EP) therefore requires the absence of certain specified microorganisms in products [1] and in raw materials (respective monographs in EP 5.1.4). Depending on the type of material, there are requirements for the absence of gram-negative rods such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella* or other microorganisms in non-sterile products. The standard test methods are given in EP 2.6.13 [2].

Testing for the absence of specified microbial contaminants, however, will not detect all potentially critical microorganisms. Due to the diversity and proven adaptability of microorganisms even to antimicrobial agents [3,4,5], this would be an unrealistic expectation. Consequently, in addition to testing for absence of certain specified microor-

ganisms the EP [6] defines quantitative limits for total aerobic microbial count (TAMC) and total yeast/mold count (TYMC).

Monitoring of Production Processes

While the pharmacopeias contain detailed requirements for the microbiological quality of the products, they provide only limited information on how this quality is to be ensured in production processes. Only the United States Pharmacopeia (USP) and the Japanese Pharmacopeia (JP) include informative chapters dealing with the microbiological aspects of production hygiene. For the manufacture of sterile medicinal products, USP 1116 [7] provides valuable information.

For the European area, more detailed specifications for good manufacturing hygiene are provided in the EU GMP guideline. In Annex 1 [8] and Annex 2 [9], control strategies to ensure hygienic conditions are described to reduce the risk of microbiological contamination. The CCS is the much-discussed core of the relatively new Annex 1, in which control measures at various levels are defined (e.g. cleanrooms classes A–D). In addition to the control measures, there are also requirements to test the microbiological quality of the

environment and to monitor the effectiveness of the control measures. One important aspect in this context is the disinfection regime for the equipment and the facilities.

Annex 1, however, does not give details on the test methods to be used. Instead, it merely formulates quantitative expectations for the microbiological situation of the environment. Microbiological test results are to be used for monitoring purposes. They are not regarded as a direct release criterion but are merely intended to indicate a possible deterioration in hygiene status including the identification of potential sources for contamination. With this knowledge, the control measures subsequently must be adapted. One example would be appropriate changes to the disinfection regime.

In contrast to the manufacture of sterile products, there are no specific requirements for microbiological environmental monitoring for non-sterile products. However, the European authorities expect such monitoring measures to be in place even in the absence of a legal requirement. In recent decades, the standards for microbiological monitoring have also been raised internationally. For example, USP 1115 at least mentions microbiological monitoring in addition to appropriate control measures [10].

Identification of Microorganisms

When it comes to identification of microorganisms, the expected depth of identification varies: For example, a distinction is made in the informational chapter USP 1113 [11] between critical and less critical findings, e.g. from Class C or D environments. For the latter, a basic characterization by microscope is considered sufficient in most cases. In contrast, a positive test for sterility or a positive Class A sample should be followed by thorough identification to the species

level. Neither the pharmacopoeias nor the GMP guidelines provide details on the identification methods to be used.

Until a decade ago, the techniques that could be used for routine identification were mainly based on metabolic reactions and staining techniques. For particularly critical isolates, molecular biological characterization (e.g. by gene sequencing) could be carried out. But for the routine identification of each organism, these techniques were far too expensive, too laborious, and could only be performed by highly qualified personnel in certain laboratories.

In recent years, the matrix-assisted laser desorption/ionization time of flight (MALDI/TOF) method has emerged as a simple, fast, cost-effective, yet reliable identification tool. This technique was a game changer, as it now allows the identification of each colony from an agar plate in routine microbiology.

Identification and contamination control strategy

When considering production processes and the effectiveness of monitoring strategies, the question arises as to whether or when the identification provides truly beneficial information. Identifying each colony down to the species level carries the risk of not seeing the forest for the trees. The goal of any effective and meaningful contamination control strategy must be to prevent microbial contamination and supply the patient with a safe product.

This places the monitoring strategy at the center of any effective contamination control strategy. Monitoring allows changes in quantity and quality of microbial contamination to be detected, so that consistent measures can be taken and followed up accordingly. Microbiologically well-qualified per-



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is Managing Director of the microbiology testing laboratory bactologicum GmbH specialized in microbiology efficacy testing. As a dedicated microbiologist with over 20 years' experience in the disinfection and technical preservation business, Katrin Steinhauer was awarded in 2020 an honorary professorship for hygiene and medical technologies at the University of applied Sciences Kiel. Katrin Steinhauer is an active member of Deutsches Institut für Normung (DIN) and the convenor of Working Group 1 in the European Standardization Committee (CEN), responsible for drafting of European Standards (EN-Methods) for efficacy testing of chemical disinfectants and antiseptics.

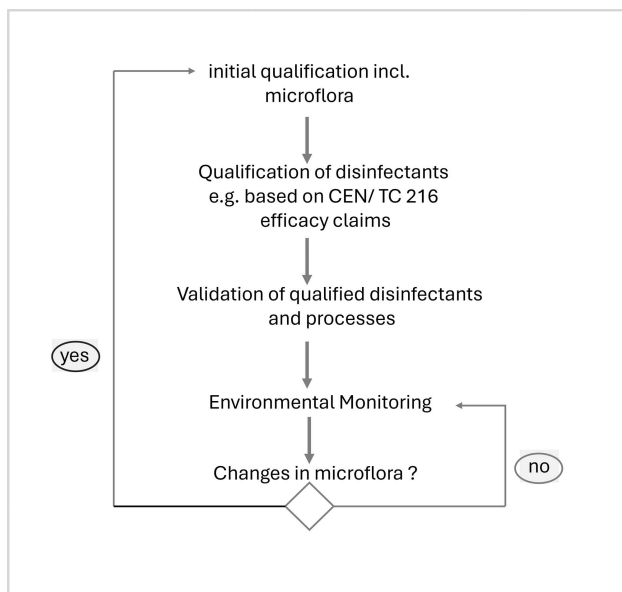


Figure 1: An effective contamination control strategy is controlled by environmental monitoring and any changes in microflora will result in requalification and revalidation (all figures provided by the authors).

sonnel can quickly and easily distinguish between gram-positive micrococci, gram-negative rods, spore-forming bacteria, molds and yeasts, even by simple methods. This information provides a quick and robust basis for qualifying suitable disinfectants based on the microbicidal claims provided by the manufacturer of the disinfectant. Typically, these claims are clustered according to the efficacy spectra defined by the methodological framework established by the European Committee for Standardization/Technical Committee 216 (CEN/TC 216) and are given in European norms (EN) for the assessment of efficacy of chemical disinfectants [12]. For example, “bactericidal efficacy” includes efficacy against gram-positive and gram-negative vegetative bacteria, “yeastocidal efficacy” against yeasts, and “fungicidal efficacy” against yeasts and molds.

Based on quite simple microbiological information, the causes of microbial contamination can be identified, and the suitability of effective disinfectants can be determined, without the need for individual colony identification down to the species level. Monitoring may for example reveal large numbers of gram-positive cocci, such as organisms of the genera *Staphylococcus*, *Micrococcus* or *Kocuria*. These genera belong to only distantly related groups of bacteria [13]. Nevertheless, detection of organisms from any of these groups would suggest the same human or airborne origin in most cases. Besides being gram-positive, these bacteria share some basic features typical for organisms that can survive on relatively dry surfaces, including human skin. A simple microscopic analysis and Gram staining can therefore be sufficient to trace contamination with these organisms back to poor hygiene practices of the staff, for example. Of course, there are

cases where a thorough identification of the colonies is crucial to pinpoint to a specific origin of a contamination. But in most cases, with this simple information, the causes of microbial contamination can be identified, and disinfectants can be qualified without the need for individual colony identification to species level and grouping of the single results.

Isolates from monitoring, such as gram-positive micrococci, gram-negative rods, spore-forming bacteria, molds and yeasts can subsequently be used to validate the efficacy of disinfection processes, which is required for the manufacturing of sterile products [8]. The validation of disinfection processes can effectively be conducted in a systematic, application-related, and needs-based manner. Figure 1 summarizes the relevant steps, starting with the initial qualification based on the relevant microflora. Thus, if e.g. gram-positive micrococci are suspected or even known to be the relevant microorganisms that need to be inactivated, this information can be used for the next step, i.e. qualification of disinfectants based on their efficacy claims. For example, a disinfectant to be qualified must exhibit bactericidal efficacy based on the qualification of gram-positive micrococci as relevant microflora in a given production process. After the qualification of a suitable disinfectant, the complete disinfection process needs to be validated, i.e. the qualified disinfectant together with its application etc.

For this purpose, relevant surfaces available on site, as well as the in-house flora (i.e. the microorganisms detected through regular microbial monitoring specifically at the production site) are used [14]. Furthermore, specific application methods, soaking volumes and cleaning equipment such as mops or wipes must be included in the validation process in order to verify efficacy. The use of these specific materials is important, as some tissues may retain active ingredients of surface disinfectants as described by Bloß *et al.* [15] for benzalkonium chloride. In addition, validating the disinfection processes also offers the potential to shorten contact times – as long as the process is proven to be robust. To achieve robust validation, di Martino *et al.* [16] based the validation of the procedures on at least 3 independent runs. The robustness of the validated disinfection process will subsequently be checked by routine environmental monitoring. Figure 2 shows an example of monitoring data.

Environmental monitoring data can be evaluated by simple means and microbiologically trained personnel can rapidly distinguish between gram-positive micrococci, gram-negative rods, spore-forming bacteria, molds and yeasts. Based on these results they can provide appropriate advice. If, for example, the limits of the “relevant” microorganisms used in qualification are exceeded as in 03/2024 in sample #3 (fig. 2), a root cause analysis needs to be conducted as part of the deviation management. With the knowledge of the causes, appropriate measures can be implemented. This may include, for example, staff training, etc.

If, as in this example, molds are detected during environmental monitoring (fig. 2, sample #1–#3, 04/2024), this finding

Site	Sample #	Limit value	01/2024	02/2024	03/2024	04/2024
Clean-room D	1	200 cfu/m ³	157 cfu/m ³ (micrococci)	145 cfu/m ³ (micrococci)	168 cfu/m ³ (micrococci)	155 cfu/m ³ (micrococci molds)
Clean-room D	2	100 cfu/4 h	55 cfu/ 4 h (micrococci)	45 cfu/4 h (micrococci)	65 cfu/4 h (micrococci)	75 cfu/m ³ (micrococci molds)
Clean-room D	3	50 cfu/plate	5 cfu/plate (micrococci)	25 cfu/plate (micrococci)	55 cfu/plate (micrococci)	15 cfu/ plate (micrococci molds)

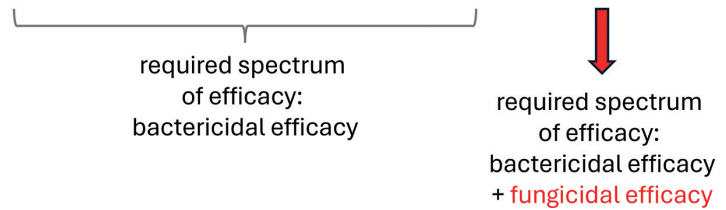


Figure 2: Environmental monitoring data can be evaluated in a simple manner. Listed are detected colony forming units (cfu).

should result in a deviation, since molds have not been originally part of the relevant microflora that was initially used for the qualification/validation. In this case, a requalification needs to be carried out, that includes molds as part of the relevant microflora. Qualification and validation steps will be adjusted accordingly, following the flowchart given in fig. 1.

Conclusion

In conclusion, even though, for particularly critical isolates, MALDI/TOF analysis or molecular biological characterization (e.g. by gene sequencing) may provide useful information, in most cases, a relatively simple characterization of the microorganisms is sufficient. A basic microbiological assessment in many cases is adequate to qualify disinfectants, validate effective disinfection procedures, to identify weak points in disinfection procedures or in human behavior, or to identify other hygiene deficiencies. Thus, even with the wide availability of high-tech identification methods, adequate microbiological training and expertise help to implement a simple, fast and sustainable contamination control strategy without the need for unconditional species-level identification of each individual colony.

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