Strategies for microbiological contamination control in Api (Active Pharmaceutical Ingredients) and finished product Manufacturing: Compliance with USFDA guidelines

Som Prakash Vats[1]*, Dr. Eshendra Kumar[2]

1 Research Scholar, Sunrise University, Alwar, Rajasthan

som_microbio@yahoo.co.in

2 Assistant Professor, School of Basic and Applied Sciences, Sunrise University, Alwar, Rajasthan

Abstract

In many cases, the quality of the finished product depends on the microbiological properties of the medicinal components. Manufacturers are expected by the USFDA to quantify and describe the bioburden of their goods. This page describes the USFDA's and compendia's concerns over microbiological contamination. Also covered in this article are ways to identify microbial contamination, how to get rid of it, and where it comes from.

Keywords: Microbial contamination; source of contamination; control of microbial contamination; USFDA concern; USP (United States Pharmacopeia) concern; microbial limit test;

INTRODUCTION

As a supplement to the USP for pharmaceutical microbiology testing which includes testing for antimicrobial effectiveness, microbial examination of non-sterile products, sterility, bacterial endotoxin, particulate matter, device bioburden, and environmental monitoring the Pharmaceutical Microbiology Manual (PMM) developed from the Sterility Analytical Manual. This manual's stated purpose is to provide ORS/CDER testing labs with a standardized framework for assessing the safety and effectiveness of medical goods in accordance with applicable scientific standards; it will do this by outlining the necessary information, procedures, and equipment. A new part of the PMM addresses inspectional guidelines for microbiologists that perform team inspections, and the manual has grown to include several quick screening methods.

LITREATURE REVIEW

Roesti (2019) With reliable microbiological monitoring and trends of data, the efficacy on the controls may be continually assessed. During the manufacture of non-sterile drug products, this chapter explains how to implement high-level multiple microbiological controls. The potential for product contamination and the various measures to lessen that risk may be determined by implementing a thorough microbial control program. A facility's environmental control systems undergo qualification as part of the facility's life cycle before operations may begin. Particularly when derived from natural sources, product components pose a high risk of microbial contamination. Process water, whether used as a solvent in non-sterile product formulations or to clean surfaces that come into touch with products, has the greatest threat of microbial contamination of any utility. Cleaning the equipment prevents non-dedicated equipment from contaminating the product and brings the quantity of bacteria down to an acceptable level.

Gupta, Rajesh. (2014). Drug and biologic stability, potency, purity, and safety may be assured with the use of microbiological approaches. In order to ensure high-quality pharmaceuticals, it is crucial to use appropriate microbiological procedures throughout manufacturing. To ensure that medications are of acceptable microbiological quality, it is necessary to use a number of overlapping procedures, including validations, monitoring, testing, and verification. In order to build a strong testing and environmental monitoring program and guarantee that the techniques are "Suitable for Intended Purpose," it is crucial to have a good grasp of the science, regulations, and difficulties associated with the design, qualification, and validation of microbiological methods. Given the complexity of emerging alternative microbiological technologies, it is vital to maintain regular discussions with regulatory bodies in order to adopt them. Improved microbiological quality assurance of pharmaceuticals and biological products may result from the simplification of technique, reduction of complexity, and variety in these procedures made possible by the use of potent contemporary technology and quick microbiological approaches.

Walsh, Andrew. (2011). As it relates to Cleaning Validation, microbiology will be covered in this chapter. Removing chemical residues, whether from active substances or cleaning agents, is the main emphasis of Cleaning Validation in practice; microbiological concerns are considered incidental. The goal of cleaning operations should never be to lower microbial residues to an acceptable level; that is, it is not their intended aim.

Ratajczak (2014) There are two categories of pharmaceuticals recognized by microbiologists: sterile and non-sterile. Pharmacopoeial monographs provide the relevant microbiological purity requirements that non-sterile medications must meet. In order to guarantee that the medication is both safe and effective for therapeutic purposes, pharmacopoeial studies are meticulously planned. The study included the outcomes of pre-marketing microbiological purity testing. The research included 1285 samples of non-sterile medications produced by several Polish pharmaceutical companies. Medications were tested for microbiological purity using standards laid forth in the European Pharmacopoeia (EP). The number of samples that did not comply was 1.87 percent, according to the findings of the tests. Medications comprising raw ingredients of natural origin accounted for 5.7% of the medication groupings that failed to meet EPs' standards. Failure to fulfill EP standards was indicated by medicine samples that included microorganisms whose presence is forbidden and exceeded the maximum permissible microbiological count limits. Twelve cases of exceeding the maximum allowed fungal count and ten cases of exceeding the maximum acceptable aerobic microbial count were the most prevalent forms of non-compliance.

Ahmed (2022) The GMP requirements are overseen by the US Food and Drug Administration, which is constituted by the Federal Food, Drug, and Cosmetic Act. To comply with these rules, pharmaceutical companies must take reasonable and effective measures to guarantee the quality, purity, and efficacy of their wares. "cGMP" is another informal way of referring to GMP. The "current" prefix serves as a reminder to producers that, in order to comply with rules, they must use modern technology and methods. It may be insufficient to test only a little portion of a batch to guarantee quality. Therefore, it is essential that pharmaceuticals be produced in a controlled environment in order to consistently fulfill the standards mandated by GMP rules. This will guarantee that quality is included into the design and production of the medicine. Our primary objective was to examine, from the perspective of pharmacists, the rationale for the pharmaceutical industry's adoption of GMP rules.

DETECTION OF MICROBIAL CONTAMINATION IN BIOLOGICAL/ STERILE PRODUCTS

The presence of microorganisms in biological products may be detected using a battery of assays. The next paragraphs detail a few of these tests.

When looking for microbiological pollutants, differential media is a lifesaver. Some of the particular media, called rich media, allow for the development of almost all microorganisms. The existence of colonies with distinct morphologies from the biological products may be used to identify microbial contamination in rich medium. Additional testing, such Polymerase Chain Reaction (PCR), may be conducted on the colonies that are thought to be problematic. Selective media are different types of media that encourage the development of certain microbes. The establishment of colonies is a way for se-elective medium to identify microbial pollutants.

Sometimes, in an effort to make the detection of microbial contamination more sensitive, the composition of this medium is changed to restrict or prevent the development of biological products. This is because there may be just a little amount of microbial contamination in the biological product, and because the development of these microbes might be hindered or reduced when exposed to high concentrations of biological product, leading to inaccurate negative findings. Sometimes, certain antibiotics, amino acids, or other nutrients are used to restrict or impede the development of the biological products.

If you suspect microbial contamination, an immunofluorescence test may help you find out. When the known microbial pollutants' type, species, and strains are known, this approach is particularly valuable since it can identify relatively low amounts of microbial contaminants. Because of this, the sponsor should verify that the test can identify cross-contaminants and commonly-known contaminants in these microorganisms before manufacturing begins.

Microbial Contamination in Nonsterile Products:

In accordance with CFR, the FDA expects manufacturers to ensure their products are free of harmful microorganisms (sometimes known as "microbial safety" or the "absence of objectionable microorganisms"). Regarding the matter of the microbiological integrity of nonsterile medications, the United States Pharmacopoeia and the United States Food and Drug Administration concur: the product need to be fit for consumption. This guarantee is well-grounded in the chapters that have been harmonized on an international level. Some revalidation of current procedures is anticipated to be necessary for the adoption of these three unified chapters. Businesses need to start making preparations for this task right now and keep proving that they are making progress. From a microbiological standpoint, the product should not be sold based on the National Formulary monograph requirement that it be free of certain

organisms. This criterion is limited at best. Chapter 1111, which deals with harmful creatures, suggests finding out how dangerous "other organisms" are, which is in line with what the FDA wants—that there be no "objectionable" organisms. While harmonized microbial limits tests do look for the "absence of specified microorganisms," it is up to each company's competent microbiology section to determine whether or not there are any objectionable bacteria.

Process controls are implemented using a microbiological viewpoint for nonsterile goods. Producing goods devoid of "objective organisms" is a clear requirement of the United States Code of Federal Regulations (21 CFR), even if these controls are not as stringent as those for aseptic production. It is important to note that the FDA's concerns on "objectionable organisms" differ from the compendial tests for "specified" species. Therefore, during the quarantine period, when the product is not being marketed, it should also be tested for the presence of "objectionable" organisms.

Table 1: List of some Drugs for APIs (drug substances) containing Endotoxin and their Limit

Drug	Limit
Amoxicillin sodium	NMT 0.25 IU/mg
Buserelin	NMT 1.00 IU/mg
Carbenicillin sodium	NMT 55.5 IU/mg
Desmopressin	NMT 500.0 IU/mg
Fosfomycin	NMT 0.083IU/mg
Heparin sodium	NMT 0.01 IU/mg
Insulin	NMT 10.0IU/mg
Oxytocin	NMT 300.0 IU/mg
Somatropin	NMT 05.0 IU/mg
Tetracyclin hydrochloride	NMT 0.50 IU/mg

Vanomycin	NMT 0.25 IU/mg
Xylitol	NMT 4.0 IU/mg

So far, only exceptional circumstances have necessitated the microbiological examination of nonsterile medications. To this day, microbiological control of these goods is essential, since very few nations have laws dictating the maximum allowable concentration of nonpathogenic microorganisms in non-sterile pharmaceuticals. The viability of such rules' implementation in GMP and the promotion of hygiene and safety must be considered in their formulation.

STEPS FOR AVOIDING MICROBIAL CONTAMINATION

In order to prevent contamination by microbes, several measures should be implemented. Environmental and cross-contamination control measures for microorganisms should be included into the facility. Secure containment of biological goods, an air pressure cascade system, numerous suites or operations for various products, and separate heating, ventilation, and air conditioning systems for each suite are all examples. Every step of the manufacturing process, including washing, formulation, and filling, must be carried out meticulously under a Class 100 laminar flow hood. It is imperative that all implements, including centrifuge bottles, glassware, growing media, and packaging materials, be sanitized before use. To guarantee that there are no contaminating micro-organisms, it is important to test the growth medium and other components involved in fermentation and production. This may be done in a variety of ways, such as using a bioburden test, sterility test, or detecting bacteria after lengthy incubation. It is important to examine the filters used to sterilize media both before and after manufacture to ensure their integrity. To detect and avoid pollutants, it is important to implement in-process controls. A possible approach to this problem is to test samples taken at various points in the manufacturing process for the presence of microorganisms that have no bearing on the biological products. Every production should conclude with a comprehensive cleaning of the production suite (e.g., using Steris(R) Corporation's LpH se(R) and Vesphene(R) IIse in a rotational disinfectant schedule), along with sterilisation of all equipment. Validated cleaning procedures should be followed to confirm the cleanliness of the rinse and swab samples. Environmental and staff monitoring of the production sites is necessary to identify microorganisms that are unrelated to the biological products. Before, during, and after every production, environmental monitoring should be carried out.

Starting materials, product-contact packaging components, production facilities, manufacturing procedures, and equipment are subject to microbiological and physical requirements and controls to guarantee that drug products are not contaminated. Controls that shield processes, equipment, and materials against potential microbiological contaminants are the main means by which these guarantees are attained. Removal, inactivation, or elimination of microbiological pollutants is also a common control method, acknowledging the limitations of preventive measures in all but the most severe cases.

It is essential that all microbiological tests be conducted in a controlled environment to guarantee that neither the product nor the material being tested is accidentally contaminated. Under these circumstances, the examination must take place in a designated space, the laboratory, which must be geographically and logistically isolated from the production areas by measures such as limiting access, providing each participant with their own air supply, and blocking the airflow between the two. Dedicated gowns and other protective apparel, a wall between the manufacturing and laboratory processes, and a system for tracking the movement of both people and materials are further precautions. Disposal of laboratory waste must be done in a way that prevents contamination of protected areas and production materials. Particular attention should be given to the design of the sampling technique. There has to be regular and suitable monitoring of the viable count in the lab. Product testing in a specific room is not allowed unless the room in question has been used for tests involving growth promotion or antimicrobial preservation, which cannot be done without a Laminar-Air-Flow Cabinet. There are a number of tests that need strict protocols to minimize the possibility of contamination, such as microbiological monitoring of purified water samples. One such protocol is the use of a Laminar-Air-Flow Cabinet.

Control of Microbial Contamination:

The main goal of microbial control of pharmaceuticals is to reduce the likelihood of contamination of medication products by microbes. Minimizing the growth of any microorganisms that may have contaminated medicinal items to a point where they pose a threat to the product's effectiveness is a secondary issue. One minor aspect of this is the testing of product samples to ensure they meet microbiological requirements. It is uncommon to find reliable statistical sampling and testing done at batch release due to the product-destructive nature of microbiological test procedures. In situations when manufacturing controls guarantee that items are exceedingly unlikely to become microbiologically contaminated, finished

product testing becomes at most confirmatory, and in the most extreme circumstances, it may even be unnecessary.

Industries that process food (including dairy), personal hygiene and beauty goods, and pharmaceuticals have protocols to prevent microbial contamination. In order to identify possible sources and pathways of contamination, it is crucial to conduct microbiological sample of products and raw materials and conduct regular hygiene monitoring of a facility and equipment. Businesses might lose money and people can get sick or die from contamination in the food industry.

An outstandingly fundamental aspect of inspection results is the control of microbial contamination. Failure to control microbiological contamination (21 CFR 211.113) was the source of eleven citations in 2005 FDA Warning Letters. These results have been included in the FDA's Top Ten List of Warning Letters for the last several years. In most situations, producing pharmaceutical items has included implementing adequate hygiene programs and practices. Control of microbiological facilities is the focus of a set of rules. The overarching objective of this system is to safeguard the pharmaceutical product against microbial contamination.

21CFR Sec. 211.113 Control of microbiological contamination states that:

Drug items that are not mandated to be sterile must adhere to certain documented protocols that aim to eliminate objectionable microorganisms.

Drug goods that claim to be sterile must adhere to certain protocols that have been devised to avoid microbiological contamination. Validation of any sterilizing process is an integral part of these processes.

21CFR Sec. 211.165 Testing and release for distribution states that:

(a) Before each batch of drug product is released, it must undergo the necessary laboratory testing to ensure it meets all final standards, including those pertaining to the active ingredient's identification and potency. Batches of short-lived radiopharmaceuticals that are subject to sterility and/or pyrogen testing may be released before the testing is finished, as long as the testing is done promptly.

(b) In order to ensure that each batch of medication product is free of undesirable microorganisms, it must undergo suitable laboratory testing, as needed.

(c) A documented protocol outlining the sample technique and the quantity of units to be tested per batch is required for all testing and sampling plans.

(d) In order for drug product batches to be approved and re-released, the quality control unit's sampling and testing acceptance criteria must be sufficient to ensure that each batch meets all relevant specifications and statistical quality control requirements. Criteria for statistical quality control must include suitable acceptance and/or rejection levels.

(e) The firm's test procedures must be proven and verified to have high accuracy, sensitivity, specificity, and repeatability. Methods for completing such validation and documentation are available under Section 211.194. (a) Part 2.

(f) Negligible drug items will be rejected if they do not match the specified standards or any other applicable quality control criteria. It is possible to reprocess. All processed materials must be completely free of defects and in accordance with all applicable standards and specifications before they can be used or accepted.

REGULATORY ASPECTS

There are times when the interests of USP and FDA align. Sterility, antimicrobial efficiency, antibiotic/vitamin potency, bacterial endotoxin, microbiological limits, etc., are all important from a USP perspective. There is zero concern for "Good Manufacturing Pro-cess" (GMP) that necessitates these testing. Substantiated in the National Formulary (NF) are the USP monographs that regulate it. To prove that a product has antimicrobial effectiveness, referees apply the "Antimicrobial Effectiveness Test" described in chapter 51 of the relevant monograph.

FDA has its own unique but related issues. Chapters listed under <1000> in USP, which deal with referees, are enforced where the requirements are equivalent. On the other hand, USP referee test methods aren't always enough to address FDA concerns. A good example of this is the need for pharmaceuticals to be "free of objectionable microorganisms" as stated in the CFR. "Appropriate written procedures, designed to prevent objectionable microorganisms on drug products not required to be sterile shall be established and followed," reads 21CFR

211.113, section "Control of microbiological contamination (a)." "Testing and release for distribution... (b) There shall be appropriate laboratory testing, as necessary, of each batch of drug product re-quired to be free of objectionable microorganisms," says 21 CFR 211.165, which supports this. Clearly, we are facing an issue. According to the current National Formulary, a product's USP monograph can state, "Absence of Pseudomonas aeruginosa." In order to prove that Pseudomonas aeruginosa is not present, you might do the test that is included in the Microbial Limits chapter. The FDA is concerned that any organisms in the finished product must be acceptable to both the product and the target population, meaning they must not be "objectionable". While this test may be necessary to show compliance with the mono-graph requirements as outlined in NF, it does not address this issue.

The FDA Concern

If your product permission to market application said that you will test the completed product by the Microbial Limits Tests, then you must actually do so; otherwise, the FDA will enforce the GMP requirement. As far as GMP is concerned, this is the only issue. Although testing according to the USP chapter may be required, it is insufficient to prove microbiological quality, and the Agency has made it quite clear that they are worried about unpleasant microorganisms in the product. The Food and Drug Administration really says this in its instructional handbook for QC microbiology lab inspections from 1993:

Topical medication treatments, nasal solutions, and inhalation products have been linked to a multitude of issues due to microbial contamination. Chapter 1111 of the USP Microbiological Attributes offers limited particular instructions, other from the following: "The importance of microorganisms in nonsterile pharmaceutical products should be assessed considering the product's use, its nature, and the possible danger to the user." Total counts and designated indicator microbiological pollutants should be regularly tested for in specific categories, according to the USP. For instance, Salmo-nella-specific natural plant, animal, and mineral items; E. coli oral liquids; P. aeruginosa and Staphylococcus aureus topicals; and yeast and mold administration articles for rectal, urethral, or vaginal use. Definitive microbiological limits are also included in a number of particular monographs. According to Dr. Dunnigan of the FDA's Bureau of Medicine, there is a health risk, and there is an acceptable degree and kind of microbial contamination in products. He warned in 1970 that gram-negative microbes in topical treatments provide a moderate to major risk to human health. Multiple illnesses have been linked to gram-negative contamination of topical treatments, according to both the

literature and our own investigations. A Massachusetts hospital reported Pseudomonas cepacia infection of Povidone Iodine products a few years ago, which is the typical case.

Hence, it is anticipated that all companies would create microbiological standards for their nonsterile goods. The technique for chosen indicator species is provided in the USP Microbial Limits Chapter <61>, just as for other undesirable organisms, but not all of them. Take Pseudomonas cepacia as an example. It's common knowledge that large concentrations of this microbe in topical products or nasal solutions are unpleasant. However, the USP does not give any test procedures to detect its presence.

The recall of the inhalation solution containing metaproterenol sulfate is an important illustration of this issue. This product does not need microbiological testing according to the USP XXII mono-graph. The presence of Pseudomonas gladioli/cepacia in the product led the FDA to classify it as a Class I recall. According to the health hazard assessment, individuals with immunocompromised conditions, cystic fibrosis, or chronic obstructive airway disease are at an exceptionally high risk of developing a lung infection, which might be fatal. The testing methods outlined in the Compendia's general Microbial Limits section would not have been able to detect these species either.

Colony identification is one possible outcome of microbial testing using the Total Aerobic Plate Count assay. It bears repeating that the USP indicator organisms should not be the only ones used for identification. The product and its intended application will determine the significance of detecting all isolates via either Total Plate Count testing or enrichment testing. Testing a solid oral dose form, as a tablet, may obviously allow for the acceptable identification of isolates when levels are high. Isolates from plate counts and enrichment tests should be identified for additional products where there is a considerable risk for microbiological contamination, such as topicals, inhalants, or nasal solutions.

The USP Concern

Proving the "absence of objectionable microorganisms" was never the goal of this chapter, according to the USP's 1982 report. "The tests described in the Microbial Limits Tests <61> were not intended to be all-inclusive, i.e., to detect all potential pathogens," the microbiology committee mentioned in a one-page Stimuli to the Revision Process stated today. This could only be achieved with a comprehensive guide on microbiological detection in the lab. Particular "index" or "indicator" or-ganisms were the intended target of the USP processes.

Although Ps. Cepacia cannot be detected in this chapter, further differentiation of the organism is necessary. This and the detection of thousands of additional potentially harmful species are also left out of the chapter's detailed instructions. Every single monograph has its own set of standards, such as maximum allowable total aerobic counts or the lack of any one of four chosen "indicator" species. To guarantee that one may test for those microbiological criteria in the specific monographs, the chapter on Microbiological Limits Tests offers techniques.

CONCLUSION

There has long been worry about the presence of unwanted and possibly harmful microbial contaminants in pharmaceutical products. The active medicinal ingredient's effectiveness, or lack thereof, might be affected by these impurities, which can have pharmaco-logical consequences that are difficult to anticipate. More worryingly, they might be poisonous. Manufacturers are compelled to use several technological and scientific procedures throughout the production cycle by the FDA's Current Good production Practices (cGMP) standards due to the grave risks posed by these contaminants to patient safety. The final purpose of these methods is to guarantee, with a high degree of certainty, that the pharmacological products fulfill all of the specified requirements. To aid in their control, compendia also provide standardized microbiological limit testing.

Abbreviation

API: Active pharmaceutical ingredient

CDER: Center for Drug Evaluation and Research

CFR: Code of federal Regulations

EP: European Pharmacopoeia

FDA: Food and Drug Administration

GMP: Good Manufacturing Practice

NF: National Formulary

ORS: Office of Regulatory Science

PCR: Polymerase Chain Reaction

PMM: Pharmaceutical Microbiology Manual

QC: Quality Control

USFDA: United States Food and Drug Administration

USP: United States Pharmacopeia

Reference

1. Ahmed, Md & Sultana, (2022). Good Manufacturing Practice (GMP) Guideline in Pharmaceutical Industries: Implementation and Its Significance from the view of Pharmacists. International Journal of Modern Pharmaceutical Research, ISSN: 2319-5878, 6(1), 46-60,

2. Ratajczak, Magdalena & Jaworska, Marcelina Maria & Kamińska, D. & Pecyna, Paulina & Dlugaszewska, Jolanta. (2014). Microbiological quality of non – sterile pharmaceutical products. Saudi Pharmaceutical Journal. 47. 10.1016/j.jsps.2014.11.015.

3. Walsh, A., "Microbial Aspects in Cleaning Validation," in Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices, Madhu Raju Saghee, Tim Sandle, and Edward C. Tidswell, eds., Business Horizons, 2011, ISBN: 978-8-190646-74-1.

4. Gupta, Rajesh. (2014). Validation of Microbiological Methods – Expectations for Regulatory Compliance. Webinar - BioPharma Asia. 3. 24.

5. Roesti, David & Goverde, Marcel. (2019). Microbiological Control Strategy. In book: Pharmaceutical Microbiological Quality Assurance and Control, WILEY, ISBN: 978-1-119-35607-3. (pp.1-21).

6. Halls, N. A. (2002). *Microbial control of pharmaceuticals*. In J. Swarbrick (Ed.), Encyclopedia of Pharmaceutical Technology (Vol. 1, pp. 1753–1764). Taylor & Francis/CRC Press

7. Fowler, J. (2005). *Monitoring your production environment*. In *Troubleshooting Microbial Contamination in an Industrial Environment* (Mould & Bacteria Reviews, Issue 11). Retrieved from

https://www.researchgate.net/publication/40999115_Microbial_Contamination_A_Regulatory_Perspective

8. Baird, R. M., Hodges, N. A., & Denyer, S. P. (2000). Handbook of Microbiological Quality Control in Pharmaceuticals and Medical Devices. CRC Press. ISBN: 978-0203305195.

9. Nighat Razvi, R. A.(2014). Estimation of microbial contamination in various API and Excipients. World journal of Pharmacy and Pharmaceutical sciences,1771-1777.

10. Chatwal, G. R., and M. Arora. *Pharmaceutical Chemistry – Inorganic (Vol. I): Impurities and Pharmaceutical Substances and Their Limit Tests*. Himalaya Publishing House, 2010, pp. 28–71.

11. Saba Sultana and Shahid Mohammed (2018). A Review on Stability Studies of Pharmaceutical Products. International Journal for Pharmaceutical Research Scholars (IJPRS), ISSN: 2277 – 7873, V-7, I-1, 2018