European Journal of Biology and Medical Science Research
Vol.12, No.2, pp.,32-51, 2024
Print ISSN: ISSN 2053-406X,
Online ISSN: ISSN 2053-4078
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development -UK

Cell Culture and Laboratory Contaminants, Prevention and Recovery Strategy- A Review

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doi: https://doi.org/10.37745/ejbmsr.2013/vol12n23251 Published September 28, 2024

Citation: Bright-Ohaeri F.C., Ihebuzo N.G., Ejeta K.O., Odimegwu N., Ifenze O. M. (2024) Cell Culture and Laboratory Contaminants, Prevention and Recovery Strategy- A Review, *European Journal of Biology and Medical Science Research*, Vol.12, No.2, pp.,32-51

Abstract: *Cell culture technology is fundamental to biomedical research, yet contamination remains a significant challenge, compromising experimental outcomes and incurring substantial costs. This review addresses key issues in current contamination management practices, including the need for customized laboratory-specific protocols, comprehensive economic assessments, and standardized recovery procedures. It evaluates methods to tackle these issues, such as automated monitoring systems, real-time contamination detection sensors, and AI-driven predictive models. Additionally, it underscores the importance of effective training programs for laboratory personnel and the value of longitudinal studies to assess the impact of low-level contamination on experimental results. By addressing these areas, the review aims to enhance the reliability and reproducibility of cell culture research and promote more effective and sustainable laboratory practices.*

Keywords: Cell culture, contamination prevention, laboratory protocols, contamination recovery, economic impact, decontamination techniques, advanced technologies, automated monitoring, real-time detection.

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INTRODUCTION

Cell culture is a cornerstone technique in biomedical research and biotechnological applications, enabling the in vitro study of cellular processes and the development of therapeutic interventions. A critical aspect of successful cell culture involves maintaining a sterile environment to prevent contamination, which can significantly compromise the reliability and reproducibility of experimental data. Contamination, whether biological, chemical, or physical, poses a major threat to cell culture systems by altering cellular behavior, skewing experimental outcomes, and potentially leading to false scientific conclusions (Bielanski, 2021). As a result, understanding the nature and impact of these contaminations, and developing robust strategies to prevent and manage them, is essential for achieving high-quality research outcomes in cell culture laboratories.
Biological contamination, the most common form encountered, can originate from laboratory personnel, contaminated reagents, and non-sterile equipment. Chemical contamination arises from impurities in culture media, plasticware, and other reagents. Physical contamination includes particulate matter such as dust, fibers, and aerosol droplets. Each type of contamination presents unique challenges to cell culture systems and requires targeted preventive and management strategies.

The consequences of contamination in cell culture are far-reaching, affecting not only the integrity of scientific research but also resulting in substantial financial losses due to wasted resources and time. Contaminated cell cultures can lead to erroneous data that misguide future research directions and clinical applications, making contamination control a top priority in laboratory management (Bielanski, 2021). Effective contamination control involves a combination of preventive measures, such as sterilization, real-time monitoring, and environmental control, as well as responsive strategies to address contamination events promptly. Establishing standard operating procedures (SOPs) and conducting regular training for laboratory personnel can significantly mitigate contamination risks and ensure reliable and valid research outcomes (Freshney, 2010).

Advanced technological solutions are revolutionizing the approach to contamination control in cell culture laboratories. Automated cell culture systems that minimize manual intervention, coupled with real-time monitoring tools employing biosensors, provide early detection of contaminants, thereby reducing the risk of culture loss and contamination spread (Yao & Asayama, 2017). Moreover, innovations in bioinformatics and machine learning are enabling predictive analytics for contamination risk assessment, optimizing laboratory workflows for contamination prevention (Jensen et al., 2020). The integration of these advanced technologies into standard laboratory practices is essential to maintaining the highest standards of cell culture research. Moving forward, the integration of automated systems and advanced analytical tools will play an important role in

redefining contamination control practices and ensuring the robustness of cell culture-based studies.

LITERATURE REVIEW

In the realm of cell culture research, ensuring laboratory integrity and preventing contamination are critical for reliable experimental outcomes. Previous works, including comprehensive reviews and empirical studies, have explored various facets of cell culture challenges, from contamination issues and preventive measures to microbial contamination and its impact on research, underscoring the need for robust protocols and advanced detection methods (Sabine et al., 2023).

A Beginner's Guide to Cell Culture: Practical Advice for Preventing Needless Problems. This review explores common issues encountered in cell culture laboratories, including cell misidentification and contamination. It provides guidelines for the prevention and resolution of these problems through a review of existing literature and standard practices. The review underscores the importance of implementing biosafety measures, aseptic techniques, and using specialized protective equipment to mitigate common cell culture problems. It emphasizes the necessity of proper laboratory protocols to prevent cell culture issues. The review does not delve deeply into specific case studies or offer detailed technical solutions for contamination issues, potentially limiting its practical application for researchers facing unique or complex contamination challenges.
(Charis-P et al., 2017). Cell Culture: Growing Cells as Model Systems In Vitro. This chapter introduces the principles of cell culture lab setup, safety guidelines, and techniques for cell propagation. It provides an overview of essential components and protocols necessary for maintaining a suitable microenvironment for cell culture. The chapter offers a comprehensive overview of cell culture components, safety measures, and general techniques. It is useful for understanding the basic requirements and practices for effective cell culture. The chapter may lack depth in specific experimental protocols and detailed solutions for contamination, which could be a limitation for researchers seeking more advanced or specific guidance.

(Laura et al., 2019). Laboratory contamination over time during low-biomass sample analysis. This study examined 144 negative control samples (extraction blank and no-template amplification controls) from both typical molecular laboratories and an ultraclean ancient DNA laboratory over a 5-year period. The study compared contaminant content between a home-made silica-based extraction method and a commercial DNA extraction kit. The contaminant taxonomic profile of the ultraclean ancient DNA laboratory was distinct from that of modern molecular biology laboratories, showing variation over time according to researcher, month, and season. The commercial DNA extraction kit showed higher microbial diversity compared to the home-made protocol. The study highlights a limited understanding of contaminant contributions in high-

throughput studies due to a relatively small number of contaminant surveys, which may affect the generalizability of the findings.

(Parth et al., 2023). Microbial Contamination of Mammalian Cell Culture. This review covers various types of microbial contamination in mammalian cell culture, including bacteria, fungi, mycoplasmas, viruses, and protozoans. It discusses methods for identification and elimination of contaminants. The review highlights the significant challenge posed by microbial contamination in mammalian cell culture, including financial loss and waste of resources. It emphasizes the need for effective detection methods and prevention strategies. The difficulty of detecting mycoplasma and virus contaminations with the naked eye presents a challenge, indicating that advanced detection methods may be necessary for accurate identification and resolution of these issues.

Historical Perspective

The concept of cell culture has undergone significant evolution since its inception in the early experiments conducted by Wilhelm Roux and Ross Harrison. Initially, these pioneering studies focused on establishing basic growth conditions for cells in vitro, marking the foundational stages of what would become a crucial tool in biomedical research. Roux's work in 1885, where he maintained embryonic chicken cells in a warm saline solution, laid the groundwork for future advancements. Harrison's experiments in 1907, which successfully cultured nerve cells from frog embryos, further demonstrated the potential of in vitro cell studies. These early efforts were groundbreaking, as they proved that cells could be maintained and studied outside the living organism. The achievements of Roux and Harrison set the stage for a century of innovation and discovery in cell culture technology (Taylor, 2014).

Over time, advancements in technology and methodology have propelled the field of cell culture forward. The development of more sophisticated techniques allowed researchers to cultivate a wider variety of cells and tissues, leading to deeper insights into cellular behavior. By the mid-20th century, techniques had evolved to support the study of complex cellular processes such as differentiation, migration, and response to various stimuli. Innovations such as the development of specialized culture media and controlled environmental conditions enhanced the viability and functionality of cultured cells. These methodological advancements enabled more precise manipulation of the cellular microenvironment, facilitating more accurate and reproducible experimental results. As a result, cell culture became an invaluable tool in biological and medical research (Rodríguez-Hernández et al., 2014).

The mid-20th century witnessed notable milestones in cell culture research, including the development of immortalized cell lines and specialized culture media. The creation of the first immortalized cell line, HeLa, in 1951, revolutionized biomedical research by providing a consistent and reliable source of human cells. This breakthrough enabled mass production of cells

for various applications, from vaccine development to drug screening. The introduction of synthetic culture media further improved the ability to maintain and manipulate cells in vitro, supporting a broader range of cell types and experimental conditions. These innovations made large-scale cell culture feasible, accelerating research in many fields. Consequently, cell culture techniques became essential for both basic and applied research (Yao & Asayama, 2017).

By the late 20th century and into the 21st century, cell culture techniques had become indispensable in biomedical laboratories worldwide. The advent of genetic engineering and molecular biology dramatically expanded the utility of cell cultures. Researchers could now manipulate cellular processes at the genetic level, allowing for detailed studies of gene function and regulation. Techniques such as CRISPR-Cas9 genome editing further enhanced the precision of genetic modifications in cultured cells. This period also saw the rise of stem cell research, where cell culture methods played a pivotal role in understanding stem cell biology and developing regenerative medicine therapies. As a result, cell culture became a cornerstone of modern biomedical research, integral to exploring disease mechanisms and therapeutic development (Farzaneh, 2021).Today, cell culture remains a cornerstone of biomedical research, supporting a wide array of disciplines, including pharmacology, toxicology, and regenerative medicine. The ability to replicate and study cellular functions outside the body continues to drive advancements in understanding disease mechanisms and developing novel therapeutic interventions. High-throughput screening methods, enabled by cell culture technologies, have accelerated drug discovery and toxicity testing. Additionally, three-dimensional cell culture systems and organoids are now being developed to more accurately model human tissues and organs. These advancements have opened new avenues for studying complex biological processes and testing potential treatments in a controlled laboratory setting. The ongoing evolution of cell culture technology promises to further enhance its applications and impact in biomedical research (Hassan & Ahmad, 2020).The historical perspective of cell culture underscores its transformative journey from rudimentary growth experiments to sophisticated techniques that underpin modern biomedical research. Each milestone in the development of cell culture methods has built upon the foundation laid by early pioneers, leading to increasingly complex and powerful tools. The evolution of cell culture has not only expanded scientific knowledge but also revolutionized approaches to studying and treating human diseases. The continued refinement and innovation in cell culture techniques are likely to drive future breakthroughs in biomedical science. Understanding the history and advancements in cell culture helps appreciate its critical role in past, present, and future research. This perspective highlights the ongoing importance of cell culture in advancing our understanding of biology and improving human health (Avar et al., 2020).

Types of Contamination

Contamination in cell culture laboratories is a critical concern, with various types posing unique challenges and risks to experimental outcomes. Each type of contamination—physical, chemical,

biological, and cross-contamination—requires specific attention to prevent compromising research integrity.

Physical Contamination involves the introduction of foreign materials into the cell culture environment. Common physical contaminants include dust particles, fibers, and aerosols, which can settle on culture surfaces or be introduced during handling. These contaminants, whether visible or microscopic, can interfere with cell growth and disrupt experimental conditions, leading to compromised research outcomes (Dervisevic et al., 2022). Examples include particulate matter such as dust, plastic, and glass debris, as well as fibers from lab attire and equipment residues. Aerosolized particles from spray bottles or during centrifugation can also introduce contaminants (Wehbe et al., 2018).

Chemical Contamination encompasses impurities in cell culture media, sera, and other reagents. Media contaminants may result from residues from manufacturing processes or inadvertent mixing of chemicals during preparation, adversely affecting cell viability and growth. Sera, particularly those from animal sources, can harbor viruses and other biological contaminants. Additionally, bacterial endotoxins found in media or culture components can exert potent cytotoxic effects, undermining cell health (Malik et al., 2023). Common sources of chemical contaminants include improperly stored reagents, errors in media preparation, and residues in sera (Ertürk & Lood, 2018; Urbischek et al., 2019; Valiant et al., 2022). Endotoxins, byproducts of gram-negative bacteria, can be highly toxic and are often found in water, serum, and culture supplements (Zandieh et al., 2018; Rasuli et al., 2022).

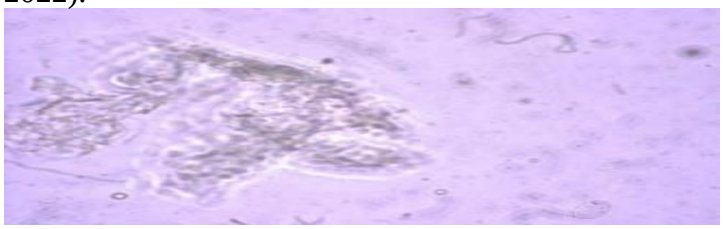

Fig. 2.1: Contaminated Fetal Bovine Serum https://www.researchgate.net/pos t/Can-anyone-tell-me-if-my-fetal-bovine-serum-is-contaminated-see-attached-pictures

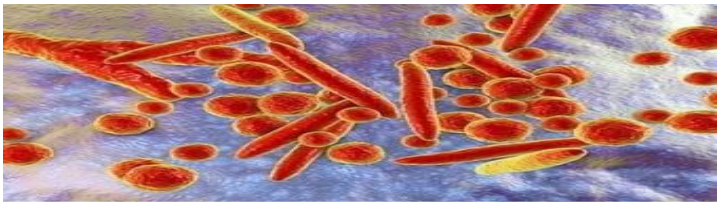

Fig. 2.2: Enhanced false color image of endotoxins

Chemical contamination refers to the presence of nonliving materials that can have adverse impacts on cell cultures. Most chemical contaminants are found in the cell culture media and result from either addition to the reagents or impurities in the water used to create the reagents.

Biological Contamination involves the presence of living organisms such as bacteria, molds, yeasts, viruses, algae, protozoa, and mycoplasmas. These contaminants are often detected by visual inspection, where infected cultures may appear cloudy or show a thin film on the surface. Biological contaminants can significantly impact cell cultures, leading to cell death or altered experimental outcomes (Srivastava et al., 2020). Examples include viral infections, which can induce cytopathic effects; insects and arachnids, which introduce microbial contaminants; protozoa, which can evade detection and resist sterilization; and mycoplasmas, which are difficult to detect and eradicate (Cheng et al., 2018; Morris et al., 2021; Zhang et al., 2019; Roingeard et al., 2019; Becherucci et al., 2021).

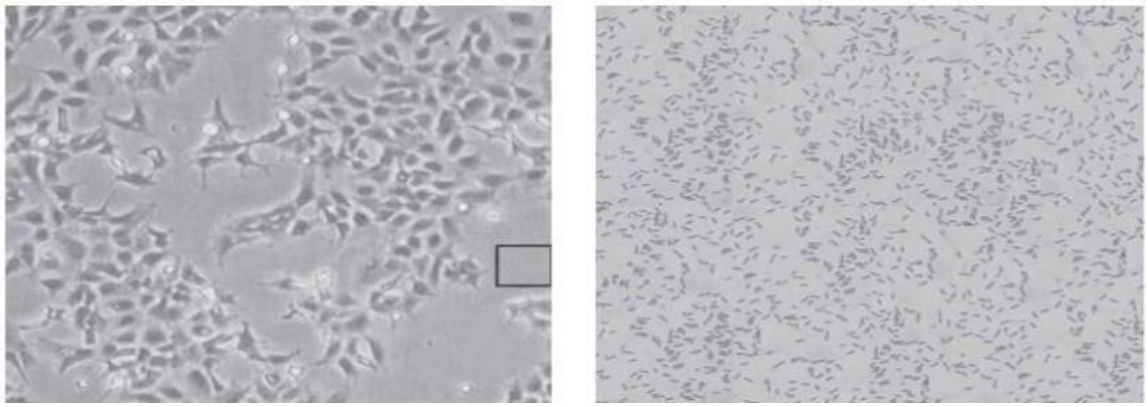

Fig. 2.3: Simulated phase-contrast images of adherent 293 cellscontaminated with E. coli. Bacteria **Source:** Managing Sterility in Animal Cell Culture Laboratory – Springer Link (https://link.springer.com/chapter/10.1007/978-3-031-19485-6_4)

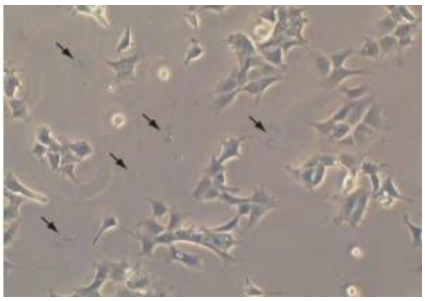

Fig. 2.4: Simulated phase-contrast images of 293 cells in anadherent culture that is contaminated with yeast. **Sources:** https://link.springer.com/chapter/10.1007 /978-3-031-19485-6_4

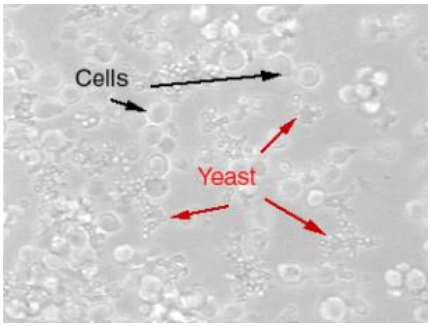

Fig. 2.5: Microscopic image showing of yeast invasion of a cell. Sources: https://unclineberger.org/tissueculture/

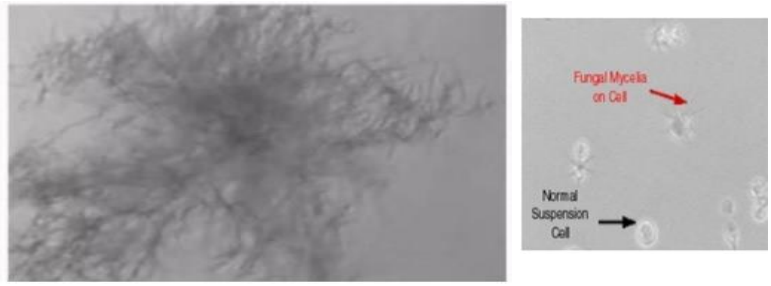

Fig 2.6: Fungal contamination of a cell
Sources: https://unclineberger.org/tissueculture/contaminant/funguscontam/

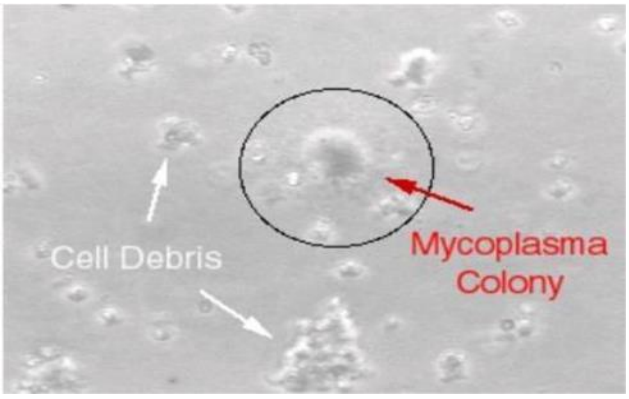

Fig. 2.7: Mycoplasma contamination Source: Mycoplasma Colonies(https://unclineberger.org/tissueculture/contaminant/mycoplasmacontam/)

Cross-Contamination by Other Cell Cultures involves the invasion of one cell culture by another, leading to a contaminated and heterogeneous culture. This issue, first identified in the late 1950s, can result in cultures being overtaken by more rapidly growing cell lines, such as HeLa cells. Cross-contamination is challenging to detect and can severely impact the validity of experimental results (Kumar et al., 2021; Janghorban et al., 2023; Vijayakumar & Sandle, 2012).

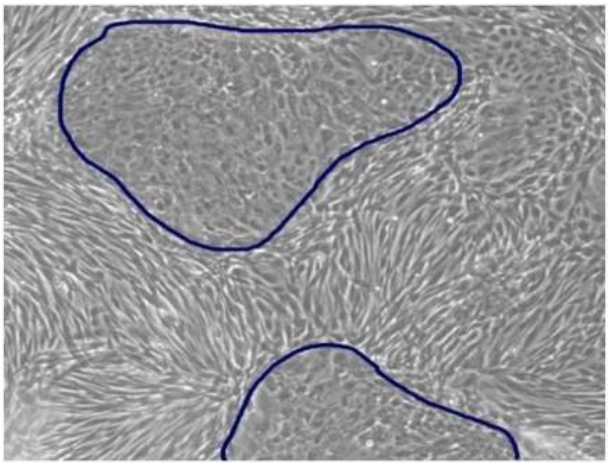

Fig. 2.8: Image showing cross-contamination with the invading cells surrounded by the blue line. You can notice the striking resemblance of the native cells.

Identification and Effects of Contamination

Identification Techniques: Identifying contamination in cell cultures requires a combination of visual inspection and more sophisticated techniques. Visible signs, such as turbidity or changes in pH, can indicate the presence of contaminants. Microscopic examination is a fundamental tool for detecting visible microbial growth (Numnuam et al., 2009). However, advanced techniques, such as PCR and sequencing, offer high specificity for identifying microbial contaminants at a molecular level (Kroh et al., 2019). Each method has its strengths and limitations, and employing a combination of approaches ensures a comprehensive assessment of contamination.

Effects of Contamination: The impact of contamination on cell culture experiments can be profound. Contaminants can alter cell physiology, resulting in erroneous data and compromised experimental outcomes. Financial implications also arise from the need to discard contaminated cultures, re-purchase cell lines, and invest in decontamination procedures (Almeida et al., 2916). Understanding the effects of contamination helps in implementing effective preventive measures and maintaining the integrity of research.

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Prevention Strategies

Personal Protective Equipment (PPE): Proper PPE is essential in minimizing the risk of contamination. Sterilized gloves, lab coats, and face masks help prevent the introduction of microorganisms into the cell culture environment (ATCC, 2021).

Sealed Culture Vessels: Using sealed culture vessels reduces the risk of contamination from airborne particles and external sources. Sealing methods and careful handling of unsealed cultures are important practices in maintaining a contamination-free environment.

Handling Practices: Avoiding mouth pipetting, using separate equipment for different cell lines, and maintaining a clean work area are crucial steps in preventing contamination. Proper handling and regular cleaning of laboratory surfaces help minimize contamination risks (ATCC, 2021).

Antibiotic Use: While antibiotics can protect against contamination, their overuse can mask underlying problems and contribute to antibiotic resistance. Careful use and monitoring are necessary to balance protection and detection of contamination (Manoharan et al., 2019).

Water Baths and Open Flames: Using clean water baths and avoiding open flames in laminar flow hoods are best practices to prevent contamination. Open flames can disrupt the airflow and compromise the sterile environment of the hood.

Labeling and Record-Keeping: Accurate labeling and record-keeping are essential for tracking and managing cultures and reagents. Standardized protocols and detailed records reduce errors and enhance communication among lab personnel.

By implementing these preventive strategies and understanding the diverse nature of contaminants, researchers can safeguard the reliability and reproducibility of cell culture experiments. Addressing contamination proactively ensures the integrity of biomedical research and advances our understanding in the field.

Table of Result and Discussion

Aspect	Results	Discussion
Customized Protocols	Laboratories that developed tailored contamination prevention protocols through detailed risk assessments reported a significant reduction in contamination incidents. Protocols were customized to address unique environmental factors, equipment, and practices.	The success of tailored protocols emphasizes the inadequacy of one-size-fits-all solutions. Customizing contamination prevention strategies improves their effectiveness and aligns them with specific laboratory needs, leading to better research outcomes.
Economic Impact Analysis	The review emphasized a lack of comprehensive economic	Understanding the economic impact is vital for justifying

	assessments regarding contamination incidents. Many laboratories failed to quantify the financial impact of contamination, such as lost research time and project delays.	investments in contamination control measures. Detailed cost-benefit analyses can help secure funding for enhanced contamination management practices, ultimately reducing long-term costs.
Advanced Technologies	The review evaluated the integration of automated monitoring systems, real-time contamination detection sensors, and AI-driven predictive models. These technologies offer continuous monitoring, immediate contamination alerts, and predictive insights.	Advanced technologies show significant potential in improving contamination management. Automated systems and sensors provide real-time data, while AI models help anticipate and mitigate risks before they manifest, leading to more effective control measures.
Training and Longitudinal Studies	Comprehensive training programs and longitudinal studies were discussed as vital components for improving contamination management. Training programs enhance staff adherence to protocols, while longitudinal studies reveal the impact of low-level contamination over time.	Effective training ensures that laboratory personnel adhere to best practices, while longitudinal studies offer insights into the long-term effects of contamination. Addressing these aspects can lead to more sustainable and reliable laboratory practices.

Enhanced laboratory-specific protocols, economic assessments, advanced technologies, and robust training are vital for improving contamination management and ensuring reliable cell culture research.

Recommendations

To address the research gaps identified in the understanding and management of contamination in cell culture laboratories, several recommendations can be made:

Future research should focus on developing and validating highly specific contamination prevention protocols tailored to diverse laboratory environments. There is a need for comprehensive economic models to quantify the long-term financial impact of contamination and justify investment in advanced control measures. Research should also explore the integration and effectiveness of emerging technologies, such as AI-driven predictive models, in real-time contamination detection. Additionally, longitudinal studies are essential to understand the subtle

effects of low-level contamination on cell culture outcomes. Investigating the development of innovative training programs and standardized recovery procedures will further enhance contamination management strategies.

Author Contributions: The author contributions for " Cell culture and laboratory contaminants, prevention and recovery strategy- a review" encompassed conceptualization, drafting, review, supervision, project administration, and funding acquisition, with each author contributing to these aspects to ensure the comprehensive coverage of the topic and the accuracy of the content.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was approved by the Institutional Review Board at federal university of technology Owerri, Nigeria.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is available upon request to the corresponding author subject to IRB restrictions and approval.

Acknowledgments: I express my profound gratitude first to God Almighty for the grace and provision to carry out this research. I also wish to specially thank my Supervisors, Prof. G.I.N Ndubuka, Engr.Dr. K.O Ejeta and Dr. Mrs.N. Odimegwu for the opportunity to conduct this research and their guidance through the period of this research "Cell Culture and Laboratory contaminates: Prevention and Recovery - A review". This endeavor has facilitated extensive literature review and has broadened my knowledge in many areas. I also wish to sincerely thank my Head of Department, Dr. K.I.N Nkuma-Udah along with all the other lecturers in the department working assiduously to impact knowledge. Finally, I like to express my heart felt gratitude to my Family.

Conflicts of Interest: The authors declare that there's no conflict of interest.

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