

## Contamination in foot-and-mouth disease vaccine production: sources, impacts, and control strategies — A smart review

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### Abstract

Foot-and-Mouth Disease (FMD) is a viral disease of cloven-hoofed animals that continues to pose serious threats to livestock health and the global economy. Inactivated vaccines continue to be the mainstay of an effective FMD control program, and much of the vaccine production relies on cell culture systems. Although improvements have been made in technology, contamination of FMD virus culture has remained a major limitation for the safety, potency, and reproducibility of vaccines. This review is a summary of current knowledge about types and sources of contamination observed in FMD vaccine production, such as bacterial, fungal, mycoplasma, and adventitious viral contamination. Aseptic handling of the product, sterilizations of equipment and reagents, reagent quality, and the manufacturing environment are highlighted, as these are common sources of contamination. The review will also address the effects contamination has on viral yield, antigen stability, and downstream vaccine quality. Moreover, the current detection, preventive, and quality control procedures at each phase of vaccine manufacture are critically reviewed. This review will focus on the existing issues and limitations in contaminant control, highlighting the areas that need to be addressed for enhanced consistency and biosafety of vaccines. We need to reinforce the overall effectiveness and reliability of FMD vaccination programs by improving contamination management.

**Keywords:** foot-and-mouth disease, FMD vaccine, microbial contamination, vaccine manufacturing, biosafety.

## Contaminação na produção de vacinas contra a febre aftosa: fontes, impactos e estratégias de controle — Uma revisão inteligente

### Resumo

A febre aftosa (*Foot-and-Mouth Disease* – FMD) é uma doença viral que afeta animais de casco fendido e continua representando uma séria ameaça à saúde dos rebanhos e à economia global. As vacinas inativadas permanecem como a principal estratégia para o controle eficaz da FMD, sendo sua produção amplamente baseada em sistemas de cultivo celular. Apesar dos avanços tecnológicos, a contaminação durante o cultivo do vírus da FMD continua sendo uma das principais limitações para a segurança, a potência e a reprodutibilidade das vacinas. Esta revisão reúne o conhecimento atual sobre os principais tipos e fontes de contaminação observados na produção de vacinas contra a febre aftosa, incluindo contaminações bacterianas, fúngicas, por micoplasmas e por vírus adventícios. São destacados o manejo asséptico, a esterilização de equipamentos e reagentes, a qualidade dos insumos e as condições do ambiente de fabricação, reconhecidos como as principais

fontes de contaminação. A revisão também aborda os impactos da contaminação sobre o rendimento viral, a estabilidade do antígeno e a qualidade final da vacina. Além disso, são analisados criticamente os métodos atuais de detecção, prevenção e controle de qualidade empregados em todas as etapas da fabricação de vacinas. Ao discutir os desafios e limitações existentes no controle de contaminantes, esta revisão identifica aspectos que necessitam de aprimoramento para aumentar a consistência da produção e a biossegurança das vacinas. O fortalecimento das estratégias de gerenciamento da contaminação é essencial para melhorar a eficácia, a confiabilidade e a segurança dos programas de vacinação contra a febre aftosa.

**Palavras-chave:** febre aftosa, vacina contra febre aftosa, contaminação microbiana, fabricação de vacinas, biossegurança.

## 1. Introduction

Foot-and-mouth disease (FMD) is one of the most economically important and highly contagious transboundary viral diseases affecting domestic and wild cloven-hoofed animals. The disease primarily affects cattle, pigs, sheep, goats, and several susceptible wildlife species, causing substantial losses through reduced productivity, restrictions on animal movement, costly outbreak-control measures, and disruption of regional and international trade. Foot-and-mouth disease is caused by foot-and-mouth disease virus (FMDV), a member of the genus *Aphthovirus* within the family Picornaviridae.

Seven immunologically distinct serotypes have historically been recognized, namely O, A, C, Asia 1, SAT 1, SAT 2, and SAT 3, and immunity against one serotype generally does not provide reliable protection against the others. The extensive antigenic diversity within and among FMDV serotypes further complicates disease prevention and requires vaccination programs to use antigenically appropriate and consistently manufactured vaccine strains (Khan et al., 2026). Vaccination remains a central component of FMD prevention and control, particularly in endemic regions and in countries implementing systematic disease-control programs. Current conventional FMD vaccines are predominantly based on chemically inactivated whole-virus antigens combined with suitable adjuvants (Gul et al., 2025; Kashif et al., 2026).

Barteling et al stated that their effectiveness depends not only on appropriate vaccine-strain selection but also on the quantity, integrity, purity, and immunogenic quality of the antigen generated during manufacturing. International animal-health standards consequently emphasize the use of vaccines produced and evaluated according to established quality requirements. Reliable vaccine production is therefore an essential element of successful FMD-control strategies, as inconsistencies in vaccine quality may ultimately reduce the effectiveness of vaccination campaigns.

The production of conventional inactivated FMD vaccines is a complex, multistage biological process in which susceptible cell cultures are used to propagate large quantities of FMDV. Cell-based vaccine manufacturing generally involves the preparation and maintenance of production cells, expansion of the cell population, virus inoculation and propagation, harvest of virus-containing material, virus inactivation, antigen concentration and purification, formulation with adjuvants, filling, and final quality-control testing. Cell culture systems, including established cell lines adapted to large-scale production, have contributed substantially to improving the scalability and reproducibility of FMD vaccine manufacture.

Nevertheless, the dependence of the manufacturing process on living cells, complex culture media, biological raw materials, specialized equipment, and repeated processing operations creates multiple opportunities for the introduction of unwanted biological agents. Research on FMD vaccine-antigen production has also highlighted contamination concerns associated with some biological culture components, including the potential introduction of bacteria, fungi, mycoplasmas, and adventitious viruses through animal-derived materials (Barteling et al., 2002).

Gaboiphiwe et al in his review stated that Contamination is therefore an important challenge in biological vaccine manufacturing and may occur at almost any stage of FMD vaccine production. Potential contamination sources include production cell substrates, seed materials, culture media, serum and other biological supplements, water systems, raw materials, reagents, equipment, processing surfaces, the manufacturing environment, and personnel. Inadequate aseptic practices, ineffective cleaning or sterilization, failures in equipment integrity, poorly controlled material transfer, and insufficient environmental monitoring may further increase contamination risks.

Because FMDV itself must be handled under stringent containment conditions, the manufacturing environment presents an additional challenge: facilities must simultaneously prevent the introduction of unwanted

contaminants into the production process and prevent the accidental escape of live FMDV from controlled production areas. International FMD laboratory biorisk standards therefore emphasize containment, controlled procedures, facility design, validated decontamination practices, and systematic risk management when infectious FMDV is handled (Gaboiphiwe et al., 2024).

According to Radlett et al. Microbial contamination may have particularly serious consequences during the upstream stages of vaccine production. Bacteria and fungi can multiply rapidly in nutrient-rich cell culture media, competing with production cells for nutrients and altering environmental conditions such as pH and dissolved oxygen. Visible microbial contamination may lead to rapid deterioration of the culture and obvious batch failure; however, low-level or slowly developing contamination may be more difficult to recognize during routine processing.

The resulting changes in cell physiology can negatively influence cell viability, metabolic activity, and susceptibility to viral infection, thereby interfering with efficient FMDV propagation. Consequently, contamination introduced early in the manufacturing process may result in reduced viral yield, inconsistent antigen concentration, increased production variability, and ultimately the loss of an entire production batch (Radlett et al., 2005).

Olarerin et al. showed that *Mycoplasma* contamination represents a particularly important concern in cell-based biological manufacturing because these organisms lack a rigid cell wall, can pass through some conventional filtration systems, and may remain undetected by routine visual examination of cell cultures. Unlike rapidly growing bacterial or fungal contaminants, mycoplasmas may persist without producing obvious turbidity while gradually altering cell growth, metabolism, gene expression, membrane characteristics, and responses to viral infection. Such hidden contamination can compromise the biological performance of production cell cultures and affect the reproducibility of virus propagation. For this reason, dedicated mycoplasma testing represents an important component of quality-control programs for cell substrates and biological products.

Adventitious viral contamination presents another distinct challenge. Unintended viruses may potentially originate from contaminated cell substrates, biological raw materials, serum-derived supplements, viral seed stocks, or other materials of animal origin. Because some adventitious agents may replicate within the same cell culture systems used for vaccine production without immediately producing obvious morphological changes, their detection may require specialized molecular, culture-based, immunological, or sequencing approaches. The risk associated with adventitious agents is particularly significant in veterinary vaccine production because biological materials may originate from multiple animal species and geographical sources. Careful qualification and traceability of starting materials, characterization of master and working cell banks, testing of virus seed stocks, and appropriate controls on animal-derived reagents are therefore critical components of contamination prevention (Olarerin-George et al., 2015).

Colenutt et al. stated that consequences of contamination extend beyond the simple presence of an unwanted microorganism. Contaminants can influence FMDV replication and reduce the amount of antigen recovered from cell culture, thereby affecting process yield and manufacturing efficiency. They may also release proteolytic enzymes, nucleases, toxins, or other metabolites capable of damaging viral structural proteins or altering the physicochemical environment required for antigen stability. Since the protective effectiveness of an inactivated FMD vaccine depends strongly on the integrity and quantity of immunologically relevant viral antigen, deterioration during production or processing may negatively affect vaccine quality and potency.

Contamination can additionally interfere with downstream clarification, concentration, purification, and inactivation processes, increase variability between manufacturing batches, complicate analytical testing, and result in costly batch rejection. The problem is further complicated by the fact that not all contaminants pose identical risks or are detected using the same methods. Gross bacterial and fungal contamination may sometimes be recognized through turbidity, abnormal cell morphology, changes in culture appearance, or conventional sterility testing. In contrast, mycoplasmas and certain adventitious viruses may require more sensitive and targeted detection systems.

Modern contamination-control programs therefore rely on a combination of conventional microbiological methods and molecular approaches, including nucleic-acid amplification technologies, alongside cell-based assays and systematic environmental monitoring. Testing only the final vaccine product is insufficient to ensure complete process control; instead, contamination monitoring must be incorporated throughout the manufacturing chain, beginning with raw materials and cell banks and continuing through virus propagation, antigen processing, formulation, filling, and final product release (Colenutt et al., 2020).

Shurson et al. stated that Prevention remains more effective than attempting to manage contamination after it has

occurred. A comprehensive contamination-control strategy requires the integration of good manufacturing practices, appropriate facility and equipment design, qualified raw materials, validated cleaning and sterilization procedures, effective environmental monitoring, personnel training, strict aseptic techniques, and well-defined material and personnel flows. Continuous surveillance of production processes can assist in detecting deviations before they develop into major manufacturing failures. Similarly, systematic investigation of contamination events, including identification of their root causes, is essential for implementing corrective and preventive actions and avoiding recurrence.

In FMD vaccine facilities, these measures must operate alongside rigorous biosafety and biosecurity systems because large quantities of infectious virus are commonly handled before the inactivation stage. FAO biorisk guidance specifically emphasizes the importance of primary containment and controlled systems when working with FMDV. Despite continued advances in cell culture technology, serum-free media, closed processing systems, rapid molecular detection, automation, and manufacturing quality systems, contamination remains a persistent concern in biological production.

The growing demand for large-scale and consistent FMD vaccine manufacturing increases the need for contamination-control systems capable of protecting both product quality and facility biosafety. However, information relating specifically to contamination across the complete FMD vaccine manufacturing chain remains dispersed among studies of cell culture, vaccine production, microbiological quality control, and biosafety. A consolidated evaluation of contamination sources, their consequences, available detection approaches, and preventive strategies is therefore important for identifying current limitations and opportunities for improvement (Shurson et al., 2023).

Accordingly, this review provides a comprehensive assessment of contamination in FMD vaccine production, with particular emphasis on bacterial, fungal, mycoplasmal, and adventitious viral contaminants. The major potential routes through which contaminants may enter cell culture and vaccine-manufacturing processes are examined, including biological raw materials, reagents, production cells, equipment, personnel, and the manufacturing environment. The review further evaluates the potential effects of contamination on cell performance, FMDV replication, viral yield, antigen stability, batch consistency, vaccine potency, product quality, and biosafety.

Current approaches for contamination detection, environmental monitoring, prevention, and quality control across different stages of vaccine manufacturing are critically discussed. Finally, existing technological and operational limitations are highlighted, together with priority areas for strengthening contamination-control practices. By integrating contamination prevention, early detection, robust quality assurance, and effective biorisk management, FMD vaccine manufacturers can improve production consistency and contribute to the supply of safer, more reliable, and effective vaccines for sustainable FMD prevention and control.

## **2. Microbiological causes of contamination**

### *2.1 Bacterial contamination*

Bacterial contamination represents a major microbiological risk during the manufacture of inactivated foot-and-mouth disease (FMD) vaccines because production depends on large-scale propagation of FMD virus in susceptible cell cultures, commonly including BHK-21 cells. The nutrient-rich media, controlled temperature, and extended incubation periods required for cell growth and viral replication can also support the multiplication of unintended microorganisms if they enter the production system. For this reason, FMD vaccine production should be conducted under sterile conditions, and the cell cultures used for virus propagation must be demonstrated to be free from contaminating microorganisms.

Bacteria may enter the manufacturing process through multiple routes, including contaminated raw materials, cell-culture media and supplements, water systems, improperly sterilized equipment, reusable processing components, air-handling systems, production surfaces, or direct intervention by personnel. Animal-derived materials are of particular concern because biological ingredients that cannot be terminally sterilized, including serum, trypsin, cell substrates, and seed stocks, may introduce viable extraneous microorganisms into the production process. Serum-containing culture systems can therefore represent an additional contamination risk, whereas carefully validated serum-free or chemically defined culture systems may reduce dependence on animal-derived materials and improve process consistency (WOAH, 2022).

Personnel-related contamination is another important consideration, particularly during open manipulations such as media preparation, sampling, transfer of cultures, connection of processing equipment, and formulation.

Inadequate aseptic practices can introduce microorganisms from skin, clothing, respiratory droplets, or contaminated gloves and tools. Similarly, failures in cleaning and sterilization procedures may allow bacteria to persist in tanks, transfer lines, valves, filters, and other difficult-to-clean locations. In large-scale bioreactor production, even a low-level contamination event may become significant because microbial contaminants can multiply rapidly under conditions originally optimized for mammalian cell growth. WHO guidance therefore emphasizes process design, facility and equipment controls, utilities, reagent preparation, operator training, environmental monitoring, closed processing systems, and validated cleaning and sterilization as interconnected barriers against microbial contamination (WOAH, 2024).

The consequences of bacterial contamination extend beyond the simple loss of sterility. Bacterial proliferation can compete with production cells for nutrients and oxygen and may alter culture pH, metabolite concentrations, and other physicochemical conditions required for optimal FMD virus replication. Severe contamination may cause deterioration of the cell culture and compromise antigen yield, whereas lower-level or slowly developing contamination may introduce variability between production batches. Bacterial cells and their metabolic products may also increase the impurity burden of harvested material and complicate downstream clarification, concentration, purification, and formulation.

From a manufacturing perspective, detection of bacterial contamination may therefore lead to rejection of intermediate material or an entire production batch, production delays, additional investigations, facility decontamination, and increased manufacturing costs. The broader implication is that contamination control is directly linked to the reproducibility, safety, and consistency of vaccine manufacture rather than being solely a final-product sterility requirement. Importantly, chemical inactivation of FMD virus should not be regarded as a substitute for effective microbiological control.

Although the virus harvest is subjected to a validated inactivation process during conventional FMD vaccine manufacture, the effectiveness of that process against every possible bacterial contaminant cannot automatically be assumed. Prevention of contamination before and during virus propagation therefore remains essential. International guidance recommends a combination of control of starting materials, aseptic processing, validated sterilization procedures, appropriate environmental controls, and testing at relevant stages of production. For FMD vaccines specifically, the WOA recommends sterility testing of the bulk inactivated antigen, concentrated antigen, and final formulated vaccine (WHO, 2016).

Traditional detection of viable bacterial contamination is primarily based on culture-dependent sterility testing. The WOA describes membrane filtration and direct inoculation as standard approaches for detecting extraneous bacteria and fungi in raw materials, master cell stocks, and finished biological products. Appropriate culture media and incubation conditions are used to recover a broad range of aerobic and anaerobic microorganisms, followed by examination for microbial growth and, where necessary, confirmation by subculture and Gram staining. Such methods remain valuable because they provide evidence of viable organisms; however, their comparatively long incubation period limits their usefulness for immediate process intervention.

Molecular methods can complement conventional sterility testing by enabling more rapid and potentially broader detection of microbial nucleic acids. Nevertheless, positive molecular signals do not necessarily demonstrate the presence of viable bacteria because residual DNA from dead or inactivated microorganisms may also be detected. Conversely, poorly designed targeted assays may fail to detect unexpected or genetically divergent contaminants. Consequently, rapid molecular methods should be validated for their intended purpose and integrated with, rather than indiscriminately substituted for, conventional microbiological testing. A risk-based approach combining culture-based methods, targeted molecular assays, environmental monitoring, and process surveillance may therefore provide stronger contamination assurance than reliance on a single final-product sterility test (WOAH, 2022).

Overall, effective control of bacterial contamination in FMD vaccine production requires a preventive, multi-barrier strategy extending from qualification of raw materials and cell banks to final product testing. Particular emphasis should be placed on validated cleaning-in-place and sterilization procedures, controlled personnel and material movement, routine environmental and utility monitoring, aseptic operator training, use of closed or functionally closed production systems where feasible, and investigation of microbial trends rather than isolated sterility results. This approach is especially important because end-product testing examines only a limited sample of a production batch and cannot compensate for weaknesses in manufacturing controls. Thus, bacterial contamination management should be embedded within an integrated quality-risk-management framework in which prevention, early detection, source tracing, and corrective and preventive actions operate throughout the entire FMD vaccine manufacturing chain.

## 2.2 *Mycoplasma* contamination

Mycoplasma contamination is one of the most challenging microbiological threats in cell culture-based vaccine production because these organisms can persist and multiply without producing the obvious signs commonly associated with bacterial contamination. This issue is particularly relevant to foot-and-mouth disease (FMD) vaccine manufacture, where FMD virus is commonly propagated in established cell lines such as BHK-21 under large-scale suspension or monolayer culture conditions. International guidance requires such production cell cultures to be free from contaminating microorganisms, as contamination of the cellular substrate can compromise the safety, purity, consistency, and reliability of the resulting biological product.

Mycoplasmas belong to the class Mollicutes and are distinguished from conventional bacteria primarily by the absence of a rigid cell wall. Their extremely small size, relatively slow growth, and close association with host cells make contamination difficult to recognize by routine visual examination. Unlike many common bacterial contaminants, mycoplasmas may not cause obvious turbidity of the culture medium or rapid destruction of the cell monolayer. Consequently, contaminated cultures can remain apparently normal for extended periods while the microorganisms spread throughout cell banks, seed cultures, and production systems. The absence of a cell wall also means that antibiotics targeting bacterial cell-wall synthesis cannot be relied upon to prevent or eliminate mycoplasma contamination. WHO consequently considers microbial agent-contaminated cell substrates generally unsuitable for the production of biological products (WOAH, 2022).

The sources of mycoplasma contamination during FMD vaccine manufacture may be diverse. Contaminated master or working cell banks can introduce the organism at the earliest stage of production and subsequently disseminate it throughout multiple vaccine batches. Biological raw materials, particularly incompletely characterized animal-derived materials, may represent additional potential routes of introduction. Cross-contamination between cell cultures, contaminated reagents, improperly sanitized equipment, and inadequate aseptic handling can further contribute to transmission within production facilities. Repeated manipulation and serial expansion of production cells increase the opportunities for adventitious contamination, which highlights the importance of using comprehensively characterized master and working cell banks and testing biological starting materials at stages where contamination is most likely to be detected.

The effects of mycoplasma infection on production cell cultures can be substantial even in the absence of readily visible cytopathic changes. Because mycoplasmas depend heavily on host cells for essential nutrients, their growth may interfere with cellular metabolism, membrane integrity, nutrient utilization, and normal cell proliferation. These alterations can reduce culture performance and potentially affect the ability of BHK-21 or other production cells to support consistent FMD virus replication. Consequently, contamination may contribute to variability in virus yield and antigen production between batches. Changes in cellular physiology may also affect experimental and manufacturing reproducibility, creating a particular concern when infected cultures are unknowingly maintained over multiple passages. Studies of mammalian cell lines have demonstrated that mycoplasma contamination can remain widespread unless active surveillance programs are implemented (WHO, 2013).

For FMD vaccine manufacture, the potential effect on antigen yield is particularly important. Efficient vaccine production depends on the generation of sufficiently high concentrations of FMD viral antigen during the cell culture phase. Any contaminant that negatively affects host-cell growth, viability, metabolism, or susceptibility to productive viral infection may consequently reduce process efficiency and increase batch-to-batch variability. Although the precise impact depends on the contaminating *Mycoplasma* species, contamination level, cell substrate, and production conditions, an infected cell culture cannot be assumed to retain the biological characteristics of a qualified uncontaminated production system.

For this reason, contamination should be regarded as a process-integrity failure rather than simply a sterility issue detected at the end of manufacturing. Detection of *Mycoplasma* contamination presents particular difficulties because conventional bacterial sterility testing alone may not provide adequate assurance of its absence. Traditional testing includes cultivation using specialized agar and broth media capable of supporting mycoplasma growth. However, some fastidious organisms, including certain strains of *Mycoplasma hyorhinitis*, may be poorly detected using culture media alone. Indicator cell culture methods combined with DNA-binding fluorescent stains can therefore provide complementary detection of non-cultivable or difficult-to-cultivate mycoplasmas. Regulatory guidance consequently supports combinations of culture-based and indicator-cell methods for reliable detection.

Nucleic acid amplification techniques, particularly conventional polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR), have increasingly strengthened mycoplasma surveillance because they can provide

faster detection than conventional culture methods. Comparative studies of mammalian cell lines have shown high sensitivity and specificity for properly validated real-time PCR methods, while PCR-based screening has also demonstrated the ability to identify contaminated cultures that might otherwise remain undetected. Nevertheless, molecular assays must be carefully validated because assay performance depends on primer design, the spectrum of detectable *Mycoplasma* species, sample preparation, amplification controls, and the potential presence of inhibitory substances. Regulatory guidance allows appropriately validated PCR-based methods when they demonstrate suitable performance relative to established detection procedures (FDA, 2010).

Rapid emerging techniques may further improve contamination surveillance. Loop-mediated isothermal amplification (LAMP), for example, has been investigated as a rapid molecular approach for detecting mycoplasma in cell cultures without requiring conventional thermal cycling equipment. Such technologies may provide advantages for rapid in-process screening; however, their use in regulated vaccine production requires rigorous validation of analytical sensitivity, specificity, robustness, and suitability for the relevant manufacturing matrix before replacing established compendial or regulatory methods.

The timing of testing is equally important. Mycoplasma control should not depend exclusively on testing the finished vaccine because contamination introduced during early cell expansion may already have affected the biological characteristics and productivity of the manufacturing process. A more effective strategy involves testing qualified master and working cell banks, virus seed materials where applicable, production cell substrates, and relevant intermediate harvests. Regulatory guidance for cell culture-derived viral vaccines specifically emphasizes mycoplasma testing at stages before extensive downstream processing or viral inactivation, thereby increasing the probability of detecting viable contamination at its source (Kazemiha et al., 2009).

Prevention therefore represents the most effective strategy for controlling mycoplasma in FMD vaccine facilities. Preventive measures should include the establishment of well-characterized and mycoplasma-free master and working cell banks, qualification of biological raw materials, segregation of untested cultures from established production cell lines, controlled personnel and material movement, validated cleaning and disinfection procedures, and strict aseptic practices. Newly introduced cell cultures should be quarantined until their contamination status has been confirmed. Routine surveillance should also be performed throughout cell expansion and production rather than only when abnormal culture behavior is observed, because mycoplasma contamination may remain visually undetectable.

The intentional use of antibiotics to suppress undetected contamination should be approached with caution. Routine antimicrobial supplementation may mask poor aseptic practices without eliminating persistent mycoplasma infection and may delay recognition of an underlying contamination event. Once contamination is confirmed in a vaccine manufacturing environment, destruction of affected cultures and re-establishment of production from a verified contamination-free cell bank generally provides greater assurance than attempting to rescue a contaminated production culture. The contamination event should also trigger root-cause investigation, assessment of potentially affected materials and production lots, intensified environmental and process monitoring, and implementation of corrective and preventive actions to avoid recurrence (Kazemiha et al., 2016).

Overall, mycoplasma contamination represents a particularly insidious risk to FMD vaccine production because it combines difficult visual detection with the potential for persistent effects on production cell physiology and manufacturing consistency. Effective control therefore requires a multi-layered strategy integrating qualified cell banks and raw materials, strict aseptic manufacturing practices, routine in-process surveillance, and complementary culture-based and validated molecular detection technologies. Incorporating rapid detection methods alongside established microbiological assays may enable earlier intervention and reduce the risk of widespread contamination. In a modern FMD vaccine manufacturing system, systematic mycoplasma monitoring should consequently be considered an integral component of quality risk management and process control rather than solely a final-product sterility requirement.

### *2.3 Fungal and Yeast Contamination*

The most common reasons for fungal/Yeast contamination of FMD cultures are poor aseptic technique, contaminated media, or poorly-sterilized animal derived components. In culture conditions, these organisms reproduce rapidly, causing turbidity, pH changes, and destruction of host cells and thereby affecting the growth of the virus. Strict sterile handling, culture material filtration, and periodic culture microscopic checking are essential to prevent such contamination. Under incubator conditions, these grow quickly, particularly in moist, nutrient-rich media, with the formation of visible filamentous networks (in case of molds) or round budding cells (in case of yeasts). As they grow, they can create turbidity, floating debris, a rapid change in the color of the culture media as

a result of metabolic waste, and eventual damage or loss of the cultured cells.

Strict sterile technique, careful reagent filtration, periodic cleaning and disinfection of incubators, proper ventilation of the laboratory, and constant microscopic surveillance to detect and prevent contamination should be used to prevent fungal contamination. Fungal and yeast contamination, in addition to competing for nutrients with the host cells, also secretes metabolic products and extracellular enzymes that may disrupt cell membranes and change the physicochemical characteristics of the culture medium (Soheily et al., 2019).

#### *2.4 Poor aseptic technique*

Aseptic handling of Foot-and-Mouth Disease (FMD) culture may permit the contamination of the culture system with microorganisms from the environment, from laboratory employees and/or from non-sterile equipment. These contaminants can increase rapidly in number, affect normal cell growth, change the environment of the cells, and affect the replication of the virus. This can result in a substantial decrease in antigen quality and yield, affecting vaccine production. If sterile techniques are not followed when handling Foot and Mouth Disease (FMD) cultures, contaminants may be introduced into the culture from the surroundings, the operator, or from tools that have not been properly sterilized.

This can result in the proliferation of microorganisms, disrupt the integrity of the cell, alter the culture environment, and impede efficient propagation of the virus. Therefore, this leads to reduced viral output and poor antigen quality, which in turn can impact vaccine production efficiency. Aseptic technique is still one of the most preventable causes of contamination in the production of FMD Vaccines. Failure to maintain good hand hygiene, wearing the wrong personal protective equipment (PPE), opening culture vessels often, and handling pipettes or culture flasks incorrectly can put microorganisms into the cell culture environment. These risks can be reduced if laboratory staff are trained regularly to master aseptic technique and follow standard operating procedures during the production process (Drexler; Uphoff, 2002).

#### *2.5 Equipment and Sterilization failures*

Contamination may occur with Foot and Mouth Disease (FMD) cultures if there are failures in equipment cleaning and sterilization, especially at the time of culture preparation or handling, which can allow bacteria, fungi, or other microorganisms to enter the culture. They may result from inadequate sterilization of media, glassware, or instruments and multiply in the culture system. Consequently, normal cell growth and viral replication are affected, with a decrease in virus yield and a decrease in culture quality. When cultures are not properly sterilized and poorly handled, the cultures can become contaminated with bacteria, fungi, or mycoplasma, which can be present in the environment. The contaminants can get into the media, unsterilized equipment, or the laboratory instruments that are used and might interfere with the growth of the cells and the reproduction of the virus, thus depressing the quality and yield of the cultures and virus (Corral-Vázquez et al., 2017).

#### *2.6 Inadequate quality control*

Poor quality control of the FMD cultures means that culture may become contaminated with bacteria, fungi, or mycoplasma without these being detected. This interferes with cell growth and with virus replication, which leads to a decrease in the amount of virus produced and a poor-quality vaccine. Poor quality control mechanisms in FMD cell culture systems can provide an environment for contamination to readily occur and proliferate. If sterility testing of culture media, reagents, and cell lines isn't done regularly, you might never notice harmful microorganisms growing in the media, and then they keep growing.

This risk is exacerbated by inadequate inspection of laboratory equipment and lack of adequate monitoring of temperature, humidity, and airflow. Furthermore, if the validation procedures are not strictly followed and critical equipment like incubators and biosafety cabinets are not maintained regularly, they can contaminate the culture environment. Procedural inaccuracies can also be caused by staff members not being trained adequately or by staff not following standard procedures. The shortcomings ultimately negatively affect cell health, viral growth, and the effectiveness and safety of vaccine production (Feng et al., 2019).

#### *2.7 Prevention and Control measure*

Careful handling under aseptic conditions, adequate sterilization of instruments and materials, and the use of

contamination-free cell lines and reagents of known origin are necessary to ensure the prevention of contamination in FMD cell culture. To minimize the risk of bacterial, fungal, and mycoplasma contamination in the culture system, all culture procedures should be carried out in biosafety cabinets under well-maintained laboratory conditions. Continuous monitoring of cultures aids in the early detection of contamination, and contaminated cultures should be discarded as soon as detected to ensure the safety and effectiveness of vaccine production (Hoff et al., 2018). Trained people, careful protocol following, and separation of laboratory activities are also essential to control of FMD virus cultures. Culture quality is maintained by regular monitoring and careful handling, and antibiotics can be used to minimize microbial growth, but good Laboratory practices are the only way to reduce the need for antibiotics.

Table 1. Identification of sources, transmission, and prevention of FMD vaccine contamination culture are given below.

| Serial No. | Source of Contamination              | Mode of Spread                                                                                 | Prevention and Control measures                                                            | References                    |
|------------|--------------------------------------|------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|-------------------------------|
| 01         | Bacterial Contamination              | Enters through Non-Sterile media, water, reagents, and pipettes                                | Use sterile media and reagents, Proper autoclaving, filtration, routine sterility Testing  | Wirth et al. (1994)           |
| 02         | Mycoplasma Contamination             | Transmitted through contaminated cell lines, sera, reagents, or laboratory personnel           | Routine mycoplasma screening, use of certified mycoplasma-free materials                   | Ishikawa et al. (2006)        |
| 03         | Yeast Contamination                  | Introduced via laboratory air, contaminated gloves, or surfaces                                | Strict aseptic technique, regular cleaning, monitoring of cultures                         | Baaity et al. (2019)          |
| 04         | Poor Aseptic technique               | Entry through improper hand hygiene, incorrect use of biosafety cabinets, reuse of consumables | Personnel training, SOP compliance, use of sterile disposable materials                    | Wehbe et al. (2018)           |
| 05         | Equipment and Sterilization failures | Inadequate sterilization due to faulty autoclave, damaged filters, contaminated incubators     | Regular maintenance, validation of sterilization equipment, cleaning schedules             | Yu et al. (2016)              |
| 06         | Inadequate Quality Control           | Delayed detection of contamination due to lack of routine testing                              | Regular sterility testing, environmental monitoring, batch documentation                   | Lawson-Ferreira et al. (2021) |
| 07         | Fungal Contamination                 | Airborne spores, contaminated surfaces, poor laboratory sanitation, open handling of cultures  | HEPA-filtered airflow, clean room maintenance, proper disinfection, minimized exposures of | Leonovich (2024)              |

Source: Authors, 2026.

## 5. Conclusions

Contamination remains a critical challenge in the production of foot-and-mouth disease vaccines because it can compromise cell culture performance, viral antigen yield, product consistency, manufacturing efficiency, and ultimately vaccine quality and safety. Bacterial, fungal, mycoplasmal, and adventitious viral contaminants may enter the production chain through cell substrates, biological raw materials, reagents, water systems, equipment, the manufacturing environment, or inadequate aseptic handling. Because many contaminants, particularly mycoplasmas and adventitious viruses, may persist without producing obvious visual changes, reliance on final-product sterility testing alone is insufficient to ensure manufacturing integrity.

Effective contamination control therefore requires a comprehensive, risk-based, and preventive approach covering the entire vaccine production process. The use of well-characterized master and working cell banks, qualified raw materials, validated cleaning and sterilization procedures, controlled production environments, closed processing systems, and strict personnel hygiene practices is fundamental to reducing contamination risks. These measures should be supported by routine environmental monitoring and systematic testing of cell cultures, virus seed stocks, intermediate materials, bulk antigen, and final vaccine products.

Conventional culture-based assays remain essential for detecting viable microorganisms, while molecular technologies such as PCR, qPCR, next-generation sequencing, and other rapid detection platforms can improve sensitivity and reduce detection time when appropriately validated. Future FMD vaccine manufacturing should increasingly integrate advanced biosensors, automated environmental monitoring, rapid molecular diagnostics, and data-driven contamination surveillance into quality-management systems. Such smart manufacturing strategies may enable earlier identification of contamination events, rapid source tracing, and timely corrective and preventive actions.

Overall, maintaining microbiological control is not merely a regulatory requirement but a fundamental component of reliable FMD vaccine production. Strengthening contamination prevention, early detection, and process monitoring will be essential for ensuring consistent antigen quality, reducing batch losses, improving manufacturing sustainability, and supporting the continuous availability of safe and effective vaccines for global FMD control.

## 6. Research gap and Future Perspective

Studies ongoing in the production of FMD vaccines focus only on a single contamination source. Still, integrated studies that standardize the study of the combination of bacterial, fungal, mycoplasma, and viral contamination on the quality of the cells in culture and the efficacy of the vaccine are not available. Research is needed to design a single monitoring and prevention strategy involving the use of more sophisticated detection techniques to reduce contamination risks and enhance the safety and efficacy of FMD vaccines.

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## 8. Authors' Contributions

*Muhammad Atif*: conceptualization, literature search, data curation, investigation, writing—original draft preparation, and review of contamination sources in foot-and-mouth disease vaccine production. *Muhammad Shahid*: methodology, validation, investigation, analysis of vaccine production processes, technical interpretation, and writing—review and editing. *Ayessha Gohar*: literature review, data collection, investigation, analysis of contamination impacts, visualization, and writing—original draft preparation. *Zainab Liaquat*: conceptualization,

supervision, project administration, validation, critical review, and writing—review and editing. *Muhammad Usman Ali Malik*: literature search, data curation, analysis of contamination control strategies, validation, and writing—review and editing.

## 9. Conflicts of Interest

No conflicts of interest.

## 10. Ethics Approval

Not applicable.

## 11. Artificial Intelligence Statement: Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors declare that generative artificial intelligence (AI) was used solely for grammatical and language editing during the preparation of this manuscript. No AI tools were used to generate, interpret, analyze, or modify the scientific content, data, results, figures, images, schemes, or tables.

The AI-assisted revisions were carefully reviewed, verified, and approved by the authors, who assume full responsibility for the accuracy, integrity, and originality of the manuscript. AI tools are not authors and cannot be held responsible for the content of this work.

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