

Contamination in a Microbiological Laboratory

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Abstract: Contamination is a clearly-established problem with serious consequences in the biological laboratory. It can be divided into three main categories (physical, chemical, and biological). The most common biological encountered contaminants are bacteria, molds, yeasts, viruses, mycoplasma, as well as cross contamination by other cell lines. This review provides an overview of major critical source and control options of contaminants. The critical source of contamination is suddenly or deliberately introduction of contaminants on the desired system during the beginning up to ending of laboratory work. To overcome the challenge numerous strategies must be daily exercised such as obtaining pure and viable cells from reputable gene banks; checking the characteristics of the culture periodically, and practicing good aseptic technique, and using antibiotics routinely. In general, this paper suggested that possible to reduce or eliminate contamination frequency and seriousness by visual inspection of the culture within a few days of it becoming infected.

Keywords: Contamination, Source, Control, and Effect

1. INTRODUCTION

Contamination is undesired introduction of impurities like chemical, microbial or physical matter, into or onto a starting or intermediating cell culture during sampling, holding, processing, storing, transferring, packaging and transporting. A cell culture contaminant is defined as some element in the culture system that is undesirable because of its possible adverse effects on either the system or its use. Contamination of cell cultures is easily the most common problem encountered in several microbial laboratories, sometimes with very serious consequences. In overall, the reason for contaminants are mostly happened through avoidable procedural errors and misguide techniques. Microbes are widely distributed in the environment without restriction and they are involved in different places even inside a laboratory. Microbial contamination is one of a biggest worldwide obstacle for researchers working with microbial cultures. It may lose laboratory valuable strains. False - positive cultures are reports of microbiological laboratory came from due to common and unusual laboratory contaminants. High microbial contaminate concentration is happened in a microbial lab due to lack of proper management. It is a global concern regarding health and leads to difficult for getting accurate research output. It is manually or systematically introduce in our culture and damage the quality of our work. Recently, many articles are mentioned it and previous reports act as evidence for the challenge. In the end, in order to reduce their first apply good laboratory practice next follow appropriate instructions [2, 6, 7 and 12].

Contaminates are receiving high attention, but the sources and processes of them are not well understood. The major methodological part of the study on the basis of an observational cohort study and long year visual investigation experience in the work area (laboratory). The aim of the current study was identified the critical sources of bacterial contamination inside laboratory then it forward answers for causes of cross-contamination how it happens? The purpose of this paper to describe appropriate and necessary options to prevent microbial, physical or chemical cross-contamination and to reduce false positive culture reports as well as to maximize the true result comes from the microbiological laboratory through practical techniques.

2. TYPES OF CELL-CULTURE CONTAMINATION

2.1. Physical Contaminants

Physical contaminants are materials consider as natural or artificial components that act as a contaminant. It also termed as unwanted foreign bodies such as glass fragments, metal, stone, plastics,

extraneous vegetable matter, hair, fibers, pipettes, storage equipment's, instruments, aluminum foil or paper residues and dust particles of incubator left by disinfectants or detergents are grouped here.

2.2. Chemical Contamination

Chemical contamination is defined as the presence of any non-living substance that results in undesirable effects on the culture system. It is useless for cell-culture at any circumstance leads to cell death. It may produce toxicity at all. Impurities found in media, sera, water, metal ions, endotoxins, free radicals, detergents, and germicides or pesticides residues are main components. In addition to this, unnecessary Impurities in gases used in carbon dioxide incubators are included.

2.3. Biological Contamination

Biological contaminants are living, subdivided into many division such as bacteria, molds, yeasts, viruses, algae, protozoa, Invertebrates, it becomes facilitated within cross contamination by other cell lines. Contamination by microbial species, which may spread via physical means (sharing media and reagents, using unplugged pipettes, improper handling and use of non-sterile reagents, accidental spilling or abnormal contact with the inanimate object) and biological means(direct or indirect contact on hands). Any types of microbial laboratories are sensitive to biological contamination. Hence, the airborne microorganisms can easily transfer, enter and outgrow desired cells in culture. The reason behind this is the presence of a high microbial load and good cultural practices are not followed [12].

3. SOURCES OF CONTAMINATION

No.	Potential source of contamination	Reference
1	<ul style="list-style-type: none"> - Field-collected sample components and sampling site. - Insufficient sample size and selection errors. - Improper sample collection and processing equipment's like plastic or metal neck tube or test tub, dissecting kit, flasks, beakers, forceps, pipettes, ice box, glass wares and laminar air flow cabinet 	[1-3, 5, 6, 8, 9-11, 13, 15-17, 19- 21, and 24]
2	<ul style="list-style-type: none"> - Excessive internal and external abiotic components (heat, cold, sunlight, moisture). - Originated from laboratory sinks, air, dust particles, benches, floors, tables, rooms, water sources, and incubator. 	
3	<ul style="list-style-type: none"> - Insect infestation, especially mites. They can move in air currents. The cultures are contaminated due to the mite traveling in and out of the culture vessels. Insects can be introduced into the lab by personnel (on hair, clothing, shoes). 	
4	<ul style="list-style-type: none"> - Use of reduced shelf-life media. -Deteriorated reagent, solution, chemicals, and water used for preparing media. -Use of materials that fail to meet acceptance specifications. -Improper calibration of utensils and equipment failure. - Supplies or resource shortage. 	
5	<ul style="list-style-type: none"> - Introduce un authorized and unskilled personnel for collection, identification, characterization and preservation of sample in all units. - Uneducated background that haven't enough ability to perform requested specific tasks. 	
6	<ul style="list-style-type: none"> - Creation of aerosol or splashing and outbreaks during specimen processing. In addition to this, Aerosols that fall out during transportation and incubation 	
7	<ul style="list-style-type: none"> - Processing excessive number of specimens in one batch due to staff shortages may lead to the breakdown in protocol adherence and invite potential error for cross-contamination 	
8	<ul style="list-style-type: none"> - Contaminates have a various way of entry into work and stock culture. Especially, it may escape from our integumentary and respiratory system due to several reasons: Inadequate personnel cleanliness, insufficient skin disinfection, and poor hand hygiene, inadequate laboratory gowning and personal protective equipment like a surgical or disposable glove and coat all these are mentioned by different researchers. 	
9	<ul style="list-style-type: none"> - Improper selecting, storing and handling of cell culture, identification instruments, electronic computer, mobile phone and other input machines. -Weak manufacturing facility. - Rough floors, walls, and ceilings. - Lack of air filtration systems. - Improper lighting and ventilation. - Poorly located vents, ledges, and drains. - Inadequate washing, cleaning, toilet, and locker facilities to allow for sanitary operation. 	

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10	- Using and disposing sharp tools for dissection and cutting broken glass, needles, or razor blades. - Chemical and heat burns or fire.	
11	- Mislabeling, poor communication, overwork due to busy work schedule. - Lack of concentration may be tension or confusion, brain and health psychological disorders. - Malpractices such as eating food, drinking beverages, or using illegal drugs.	
12	- Impurities in gases used in carbon dioxide incubators.	
13	- Misguided study design, experimental set up and culturing protocol. - Out of the scientific basis of investigation and follow-up. - Undesired spend time and energy for laboratory trials.	

4. THE CONTROL MECHANISM OF CONTAMINATION

No.	Control mechanism	Reference
1	- Follow your facility's written policy. - Don't pour chemicals down the drain. - Cleaning and decontaminating should be performed at the ending and beginning of the day.	[1, 2, 4, 6, 10, 11, 13, 14, 18, and 23]
2	- Apply sterilization methods like autoclave at 121°C for 20 min and hot air oven at 160°C for 1 h, micro or ultra-filtration, incinerator, ultraviolet rays, flame, formalin, and ethanol. -The use of sterile equipment's and environment helped to prevent further contamination of the given sample. Even materials are covered through sterile aluminum foil have a crucial role in the quality of research outcome.	
3	- Maintain laboratory environment. Firstly, exposed for fumigation. Secondly, continued air sampling for microbiological evaluation. Thirdly, adjusted all laboratory physical factors (Attain room ventilation system that replaces fresh air in air filtration path), Fourthly, free from human unwanted influence and disturbance finally reduce opportunities for an accident.	
4	- Make sure that both media bottles and flasks are dry and wiped with 70% alcohol or 5% sodium hypochlorite before they are available for further preparation and putting into the hood. - Avoid leaving bottles opened for a long time which contain (media, solutions and so on) close after use it immediately.	
5	- Proper instrument installing, packaging and handling - Checking validation date of items.	
6	-Work carefully in aseptic condition at the time of sample collecting and processing. -Clean the work area with disinfectant to prepare a safe and sterile workspace: <ul style="list-style-type: none"> • Specimens and pouches should be handled one-at-a-time. • Sample decontamination • Handling of only one cell line at a given time. 	
7	- Reduce the opportunity for cell culture accidents the main possible solutions are assigning with clear label of color ink, a written procedure, good supervision, assigned tasks, careful planning and, backups	
8	- Follow up inoculation and other aseptic procedures. - Aseptic transfer of cultures and sterile solutions. - Preparing inoculants and cultures for study use. - Use stock culture for short and long term and kept in appropriate temperature as well as test the stock for sterility before reviving. - Working with only one cell line at a time in order to prevent cross- contamination effectively. - Do not leave feeding culture on lab benches for a long time. - Sealing culture vessels must be done. If an insect problem is in progress to control it by using insecticides, plaster and plastic box. Commonly, Parafilm is used to wrap culture vessels. - An observational screen of viable cells regularly and check the characteristics of the cell lines periodically for the presence of contamination. Screen all types of new cells as they enter the lab, before and after cells are thawed, it is important to select unrequired suspects before the paper publicized. - An additional subculture of mixed and contaminated bacterial culture by using numerous techniques or else discard contaminated culture.	

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9	<ul style="list-style-type: none"> - Proper pipetting technique ensures that the accurate volume is aspirated and dispensed and avoids splashing when dispensing liquid. - Keep reactions and components capped whenever possible
10	<ul style="list-style-type: none"> - Using selective and have shelf-life media for preparation and further tasks. - Never store media and organisms in cold facilities with light. - Addition of growth inhibition substances in the medium
11	<ul style="list-style-type: none"> - Aerosols can lead to cross-contamination from sample-to-sample. - Aerosol-resistant pipette tips have a barrier, which acts as a seal when exposed to potential liquid contaminants, trapping them inside the barrier.
12	<ul style="list-style-type: none"> - Training personnel for a relevant specific target of decontamination protocols. - Providing extra time for each lab lesson and spend a lot of time protecting themselves inside the laboratory. - Always wear personal protective equipment. Wear full sleeved coats, such that the coats are kept inside the culture room and worn only after having a good ultra-violet bath for at least 30 minutes. - Unidirectional workflow with personnel and material.
13	<ul style="list-style-type: none"> - Keep work surfaces and materials neat and orderly - Avoid clutter. - Use small containers when possible - Return reagents and others in its original place after finished activity - Clean up facility should be performed routinely. This includes floors, ceilings, and walls - Empty bins regularly - Clean any spills immediately. Spills should always decontaminate the area though follow proper disposal procedures - Remove and decontaminate all unnecessary equipment after using it and store appropriately - Check surfaces and tools before, during and after work periods. - Document the cleaning status of each equipment in logbooks
14	<ul style="list-style-type: none"> - Always wear sterile gloves when working in the collection, transportation, isolation, identification, characterization of the sample. - Change gloves frequently, especially in DNA and RNA amplification on each specimen - Basically, try not to handle anything without gloves,
15	<ul style="list-style-type: none"> - Microbial laboratory set up is designed specifically that suitable for scientific experiments. Written laboratory records for every cell culture.
16	<ul style="list-style-type: none"> - Use a standard surgical mask to protect active respiratory track oral droplet direct contact. - should avoid touching the mask while preparing specimens. - Do not talk inside the culture room. If so, use masks. Your mouth has fungi that can be a possible source.
17	<ul style="list-style-type: none"> - Spray over your hands with appropriately diluted alcohol (70%) up to your elbows, during cell handling. - Wash hands thoroughly with antiseptic soap and warm water before entering and leaving the laboratory. The control of all types of contaminations start from personal hygiene of hands and travel through all steps of culture.
20	<ul style="list-style-type: none"> - Avoid culturing of animal, human and microbial cell together within the same incubator. - Incubators are to be cleaned with recommended solution. It may be daily, weekly and monthly.
21	<ul style="list-style-type: none"> - Attain room ventilation system that replaces fresh air in air filtration path. - Air filtration and air change rates should be set to ensure that the defined clean room class is attained. - Airflow over critical areas should be uni-directional (laminar flow) at a velocity sufficient to sweep particles away from filling/closing area. - Air handling ducts should be cleaned and properly maintained and pre-filters venting the air into the culture room should be replaced frequently
22	<ul style="list-style-type: none"> - When entering the cabinet, wipe down the surface with 70% ethanol - Allow the air in the hood to circulate for 5 minutes before use. This will allow any contaminants to hopefully enter the laminar flow and not end up in your media or dishes.

<ul style="list-style-type: none">- Used for performing viral or bacterial culture should not be used for specimen preparation or pouch loading- Place closed samples and processing supplies in the hood and expose to UV lamp for 15 minutes- After sample processing is complete, wipe down the surface again with 70% ethanol- Close UV shielding, and lamp automatically comes on for 15 minutes <p>Laminar Air Flow Hood: 1. dos and 2. don'ts</p> <p>1. DO</p> <ul style="list-style-type: none">-Keep the cabinet fully closed when not in use; Always wear gloves; Work at least 6" inside the cabinet; keep head out of the cabinet <p>2. DON'T</p> <ul style="list-style-type: none">-Create significant turbulence in the cabinet with rapid motion; Store anything in the hood because it can block sterile air flow and will compromise sterility; Sneeze or cough in the direction of the hood; Operate the hood with UV on and the cabinet open; Avoid making fast movements in and out of the hood.	
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5. EFFECTS OF CONTAMINANTS ON CELL/MICROBIAL CULTURE AND OTHERS

- Competing for nutrients and they are hinders for cell growth and proliferation
- Expositing cells to unwanted primary and secondary metabolites utilization and production respectively.
- Altering levels of protein, RNA, or DNA synthesis in terms of quantity and quality
- Changing gene expression, cell signaling, morphology and physiology
- Damaging membranes and organelles at the high level
- Causing mutations and chromosomal changes
- Destroying the natural microbial community structure and function
- Devastating the growth and characteristics of the cultures. It has adverse effects on inhibition of cell metabolism
- Damaging of valuable products and boring to get pure and viable culture.
- Contributing several risks /outbreaks of laboratory - acquired infections for technicians, researchers, healthcare workers and patients. Immune deficiency individuals are sensitive to severe human disease. The persistent pathogens the mode of transmission is huge in number
- Influencing on signal transduction
- Serving as public health concern it becomes available for a biological weapons.
- Causing severe economic challenges registered
- Losing the quality of research outcome and inaccurate or erroneous experimental results recorded
- Losing time, money (for cells, culture vessels, media, and sera), and effort (spent developing cultures and setting up experiments) already happen
- Frustrating feelings become occurred and Personal embarrassment also.

To summarize, the three best practices to maintain the integrity and accuracy of cell cultures, and promote a safe laboratory environment. First, use appropriate lab design. Second, use correct culturing procedures. Thirdly, use suitable cleaning procedures. It is the best guarantee for successful completion.

6. THE FUTURE DIRECTION OF CONTAMINATES

Laboratories require investigation review process to identify unusual numbers of false-positive cultures and a mechanism to determine the possible causes profoundly. As a minimum, it must be conducted through monthly or annually follow up period. Continuing communication among clinicians, laboratory technicians and researchers remains critical in the interpretation of unusual findings that develop from cross and self-contamination. In the future, three activities are attentively supported. That is: (I). Identity of the taxa through various well accepted scientific methods like metabolic and genetic fingerprinting.

- (II). Sources of potential hazards based on the isolation and identification of indicator microorganisms,
- (III). Development of methods for the treatment of microbiological hazards.

When starting to explore contaminants. It will be promising for further outcomes such as antibiotic production, medical value, biotechnological research and antimicrobial activity for drug resistance and susceptibility assays. In the future, It is very important to generate full scientific knowledge through continuous research even can make a survey in the field of microbial diversity (community, functional and structural) pattern. Additional systematic studies will be performed in the area in order to ignore frustrating and devastating idea: To accomplish unique and get suitable viable pure culture. The merit will be greater than the disadvantage part of it.

7. CONCLUSION

Generally, the present study reported that laboratory equipment and environment act as the potential sources of contamination which exhibit high concentration. To solve the problem various methods must be applied. Nowadays, Formulate, implement and revalidate protocols at regular intervals are most acceptable and affordable option. Establishment of control points is essential for all. Appropriate and careful management strategy considering as a critical points step by step starting right from collection of sample to their processing can significantly minimize contamination load. Lastly, concluded that contaminants can cause loss research work and interfere with the normal arrangement or functioning of central cell dogma. For this evidence, this paper helps to address short and long-term effect of contaminants in the microbial laboratory. To sum up, contamination can be totally eliminated, it can be managed to reduce both its frequency of occurrence and seriousness of its consequences by introducing good laboratory practice.

RECOMMENDATION

Always seasonal survey and continued surveillance should be conducted on physical, chemical and microbial assemblages to maintain the purity of culture. Practical based subsequent control mechanisms should be deemed to remove the risk of a cross-contamination.

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