REVIEW

Contamination development, detection and disposal in animal tissue culture

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The use of animal and human cell cultures has become very beneficial for diverse applications in biotechnology and biomedical research. Originally used as substrates for the production of viral vaccines, animal and human cell cultures became an indispensable tool to produce a variety of products, including biopharmaceuticals, monoclonal antibodies and products for gene therapy. The use of animal and human cell cultures also constitutes adequate test systems for studying biochemical pathways, virus production, pathological mechanisms or intra- and intercellular responses. All cell culturist encounter contamination at some point in their work. One can avoid getting contamination but if that is not possible then one must destroy all contaminated cultures. Contamination in cell culture influences with parameter of cells under study. Therefore risk assessment should result in the implementation of appropriate containment measures and work practices in order to provide maximal protection of human health and environment and avoid the contamination.

Types of Contaminants

Contaminants that you can see

These contaminants are easiest to prevent, detect and to deal with.

Bacteria and fungi

They are the most common forms of contaminants in cell culture.

They are frequently air-borne and result from improper sterilisation, storage of reagents and materials and poor aseptic techniques.

Bacteria and fungi (including yeasts and mold) are too small but they turn the medium cloudy and frequently turn the pH of the medium to acidic levels with their metabolic by products.

They are easily visible using phase-contrast microscopy and less difficult to deal with.

Bacteria

In general, bacteria do not attach to the cells and are dispersed in the medium appearing as specks in contrast to adherent cells.

Bacteria have a size of around 1 mm therefore appear smaller than non-adherent cells and can be easily detected under microscope. To isolate the bacteria take a small portion of culture medium, Centrifuge, Resuspend the pellet in an isotonic solution. Smear the sample on a glass slide and stain with acid fast or gram stain and view under oil immersion lens for specks. If dark motile specks are observed then immediately dispose of the culture.

Fungi

Two different forms of fungal contamination can be distinguished from the cells, the Yeast form and the mold form.

Yeast form appears as refractile circles floating in the medium or as loosely associated with the cells. They generally have a size of 2-5 mm and hence can be distinguished from bacteria, which are much smaller in size. The floating cells appear much larger than the yeast form in the medium making the detection easier.

Mold form appears as long filaments that are intertwined. They generally have a diameter of 5 mm. Their hyphae block transmission of light and mold is seen as a dark central area with extended hyphae.

Detection

If the cultures are identified as acidic, look cloudy, have fluffy balls or have strings in them, then remove these from incubator and observe under the microscope.

Precautions and handling

Never open the contaminated culture dishes as they may contaminate other dishes.

If there is a contaminant in the culture, tape the tissue culture dish closed, place it in a sealed bag and autoclave. Wipe down the microscope stage, bench top and hands with alcohol.

Mold can grow on outside of culture dishes and on plastic, metal, glass surface and paper labels.

Yeast, mold and bacteria are ubiquitous and are found adhering to dust and water vapour in air, hair, skin, clothing and shoes.

How to avoid contamination?

Place the contaminated dishes in a separate autoclave bag and remove from room for sterilisation

Filter the air in the room with a HEPA filter and maintain the optimum temperature with a thermostat system.

A sticky mat placed inside the entrance door to the culture room removes dust and spores from shoes of people entering the room.

Lab coats must be worn in culture room.

Locate the sink adjacent to culture area entryway so the culturists wash hands prior to entering culture room.

Contaminants that you cannot see

Mycoplasma

Compared to bacterial or fungal infections, contaminations with mycoplasma give more problems in terms of incidence, detectability, prevention and eradication. Mycoplasma infection may go undetected for many passages, causing a variety of unpredictable effects causing harm to the host cell. Mycoplasma infection may also influence the sensitivity of host cells for growth of viruses.

Mycoplasma are smallest organisms known to exist as free cells. They lack cell walls. They are known to be present in cultures containing penicillin and streptomycin.

They can exist as single cells and also form colonies and exhibit a fried-egg like appearance on agar. Aerosols are regarded as the major source of mycoplasma contamination of tissue cultures (O'Connell *et. al.,* 1964). Mycoplama have slow generation time and therefore are not easily visible. They don't directly destroy cells but alter function and metabolism of cell culture, cause chromosomal aberrations, interferes with nucleic acid synthesis, degradation of the cell DNA and morphological transformation (Macpherson, 1966). Mouth pipetting should be avoided in the culture room as a precautionary measure as mycoplasma spread by respiration.

Detection

If contamination is suspected in culture and medium then Basic dye Giemsa stain is used for light microscopy, which binds to the DNA in culture and mycoplasma Therefore, contaminated cells display intense localised staining inside and outside the nucleus whereas uninfected cells will only show intense staining within nuclear borders. In case of fluorescent microscopy, stains like Hoechst H 33358 dye is used which bind mainly to the DNA so mycoplasma containing DNA are seen as bright punctuate staining in cytoplasm.

Viruses

They are too small to observe with light microscopy. They cannot replicate outside the host cells and result in intracellular contamination. Viral contamination needs particular attention because infection may be without cytopathic effect for the cell culture or may be latent (e.g. herpesvirus) and hard to detect. Human and non-human primate cultures are more likely to harbour viruses that are highly pathogenic to humans. Of particular concern are the blood-borne viruses such as Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and others such as Hepatitis C virus (HCV) and Human T-cell lymphotropic viruses (HTLV). However, non-human cell cultures are not without risks as they also contain viruses with a broader host range able to infect humans such as rodent cell culture carrying hantavirus (Lloyd and Jones 1986) or primate cells harbouring Marburg virus.

Detection

Endogenous and contaminated viruses present in cell culture systems are difficult and expensive to identify. Endogenous retroviruses are undetectable in cell culture except by serological and biochemical methods. Their presence is detected by a change in normal properties of culture such as increased debris in culture, cell death, alteration in growth. Generally, fluorescent or transmission electron microscopy is employed for their detection. Recently improved methods such as Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and RT- nested PCR have been used for detection of viruses (Johnson *et. al*., 2001).

Safety measures

Handle the cells in a hood with HEPA-filter

Autoclave cell culture waste, treat spent media with a viricide.

Test primary tissues for hepatitis and HIV when they are removed form donor.

Parasites

Adventitious contamination with parasites may be an issue when handling freshly prepared primary cell cultures or tissue cultures, if those are originating from a donor organism which is known or suspected to be infected with a specific parasite. Well-known intracellular protozoan parasites for which laboratory-acquired infections have been reported are *Toxoplasma gondii*, *Trypanosoma cruzi*, *Leishmania sp*, *Cryptosporidium parvum* , *Plasmodium sp*. etc. (reviewed by Herwaldt *et. al*., 2001).

Prions

Proteinaceous infectious particle (Prion) is an infectious agent comosed only of protein. Prinons cause a number of diseases in a variety of animals and in humans. Though a limited number of cultured cell lines (e.g. mouse neuroblastoma cell lines Sc N2a) have been shown to promote, upon sub-passaging, stable and persistent replication of PrP(Sc) as well as infectivity (Solassal J, *et. al*., 2003), most cell lines are resistant to prion infection (Butler, *et. al*., 1888). However, in contrast to most of the infectious agents, prions are particularly difficult to inactivate. In fact no method can guarantee total inactivation of these agents. So, one should bear these considerations in mind when using growth media of bovine origin.

Cross-culture contamination

It is the most difficult type of contamination in which cell type is contaminated by another cell type.

Cross-contaminated cell lines can be carried as mixed cultures for long periods of time or the contaminating cell may take over the entire culture causing loss of original cell line. Detection becomes difficult if the cell lines are similar in appearance and derived from the same species (Mather and Roberts, 1998)

Precaution to prevent cross-culture contamination

- A new cell line in the laboratory must be typed to determine its identity.
- Carry out an isozyme typing to determine species of origin of the cell line.
  Karyotyping also provides information on species of origin.
- Don’t use two different cell lines in the hood at the same time.
- Don’t put same pipette tip into two sequential culture stock.
- Wipe down the hood surface, wash hands and change gloves, between handling

different cell lines.

- Store frozen cells in vapour phase of liquid nitrogen or use glass freezing vials.
- Despite of all these precautions if the culture is still cross- contaminated then destroy it.

General methodology for dealing with contamination

Once a culture is identified as contaminated, it should be disposed of as early as possible. Some researchers have tried out curing the contaminated cultures with treatment of antifungal, antiviral reagents or antibiotics but such attempts should be avoided as such additions may be harmful to the cultures (Martin, 1994). In routine practice, a contaminated culture should be taken from the culture area without opening the culture vessels. Then a strong disinfectant is added to the cultures. Pour of the excess disinfectant and then rinse the vessel with water and discard. Alternatively, the contaminated cultures can be placed in an autoclave bag and directly autoclaved before disposal. Never autoclave the cultures with disinfectants present, to avoid the release of toxic by-products. The culture incubator should also be thoroughly cleaned with disinfectant, rinsed with water, then with ethanol and dried. After this, wipe areas exposed to the contaminated cultures.

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