

Incubation - Contamination Control



make sure the correct gas cylinder is used. Other potential chemical contaminants are the toxic, volatile residues left behind after cleaning and disinfecting incubators. Disinfectant odors should not be detectable in a freshly cleaned incubator when it is placed back into use.

BIOLOGICAL CONTAMINATION
Biological contaminants can be subdivided into two groups based on the difficulty of detecting them in cultures: those that are usually easy to detect — bacteria, molds and yeast;

- those that are more difficult to detect, and as a result potentially more serious culture problems, — viruses, protozoa, - insects, mycoplasmas and other cell lines.

BACTERIA, MOLDS, AND YEASTS
Bacteria, molds and yeasts are found virtually everywhere and are able to quickly colonize and flourish in the rich and relatively undefended environment provided by cell cultures. Because of their size and fast growth rates, these microbes are the most commonly encountered cell culture contaminants. In the absence of antibiotics, microbes can usually be readily detected in a culture within a few days of becoming contaminated, either by direct microscopic observation. (See Figures 1 and 2.) or by the effects they have on the culture (pH shifts, turbidity, and cell destruction). However, when antibiotics are routinely used in culture, resistant organisms may develop into slow growing, low level infections that are very difficult to detect by direct visual observation. Similar detection problems can occur with naturally slow growing organisms or very small or intracellular bacteria that are difficult to see during routine microscopic culture observation. These robust contaminants may persist indefinitely in cultures causing subtle but significant alterations in their behavior. By the time these cryptic contaminants are discovered, many experiments and cultures may have been compromised.

Sanyo SafeCell™ UV and inCuSafe™ contamination control technology offers efficient and proactive as opposed to reactive strategies for managing contamination to prevent the loss of valuable cultures and experiments.

Contamination Control CELL CULTURE

The most obvious consequence of cell culture contamination is the loss of researchers time, money (for cells, culture vessels, media and sera) and effort spent developing cultures and setting up experiments.

Table 1. Some Consequences of Contamination

- ▶ Loss of time, money, and effort
- ▶ Adverse effects on the cultures
- ▶ Inaccurate or erroneous experimental results
- ▶ Loss of valuable products
- ▶ Personal embarrassment

However, the less obvious consequences are often more serious (Table 1). First there are the adverse effects on cultures suffering from undetected chemical or biological contaminants. These hidden (cryptic) contaminants

can achieve high densities altering the growth and characteristics of the cultures. Worse yet are the potentially inaccurate or erroneous results obtained by unknowingly working with these cryptically contaminated cultures.

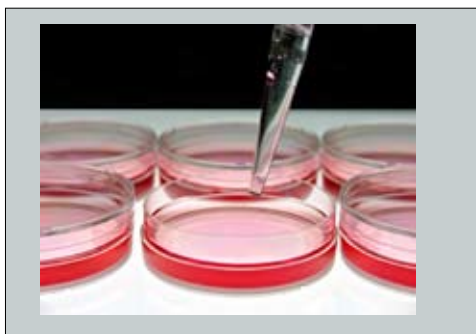
Products, such as vaccines, drugs or monoclonal antibodies, manufactured by these cultures will probably be useless. For some researchers the most serious consequence of contamination is suffering the embarrassment and damage to their reputation that results when they notify collaborators or journals that their experimental results are faulty and must be retracted due to contaminants in their cultures.

Preventing all cell culture contamination has long been the dream of many researchers, but it is an impractical, if not impossible, dream. Contamination cannot be totally eliminated, but it can be managed to reduce both its frequency of occurrence and the seriousness of its consequences.

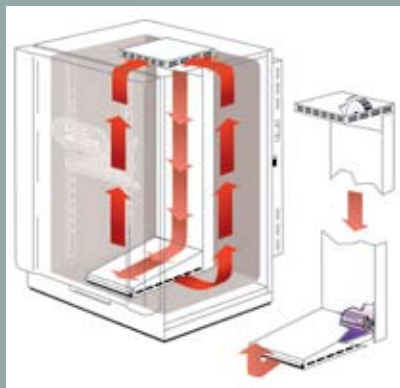
INCUBATORS

The incubator, often considered a major source of biological contamination, can also be a source of chemical contamination.

The gas mixtures (usually containing carbon dioxide to help regulate media pH) perfused through some incubators may contain toxic impurities, especially oils or other gases such as carbon monoxide, that may have been previously used in the same storage cylinder or tank. This problem is very rare in medical grade gases, but more common in the less expensive industrial grade gas mixtures. Care must also be taken when installing new cylinders to



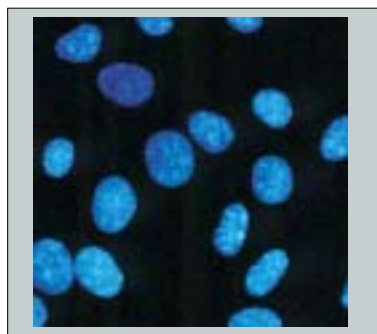
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Sanyo SafeCell™ UV and in-CuSafe™ contamination control technology offers efficient and proactive as opposed to reactive strategies for managing contamination to prevent the loss of valuable cultures and experiments.

SafeCell™ UV Series CO2 incubators include contamination control technology based on an integrated combination of narrow bandwidth, ozone-free ultraviolet light, ceramic infrared CO2 control, inCusaFe™ copper-enriched stainless steel alloy interiors and Direct Heat and Air Jacket™ heating managed by a microprocessor controller. These incubators are useful for the most critical applications where continuous contamination control is essential to cell viability.

In most laboratories, the greatest sources of microbial contamination are airborne particles and aerosols generated during culture manipulations. The microbial laden particles are relatively large (generally 4 to 28 µm in diameter) and settle at a rate of approximately one foot per minute in still air. As a



result, the air in a sealed, draft-free room or laboratory (no people, open windows or doors, air handling units, air conditioners, etc.) is virtually free of biological contaminants. However as soon as people enter the room, particles that have settled out will be easily resuspended. In addition certain equipment and activities can generate large amounts of microbial laden particulates and aerosols: pipetting devices, vacuum pumps and aspirators, centrifuges, blenders, sonicators, and heat sources such as radiators, ovens, refrigerators and freezers.

In light of this major frequency and occurrence of airborne contamination within laboratories Sanyo has developed a continuous Active Background Contamination Control™ process that eliminates contamination without downtime. Contaminants trapped within the distilled water pan are destroyed by ultraviolet light.

Sterile, humidified air is released from the lower plenum for vertical convection through and around the perforated shelves. Interior air motion is suspended when the door is opened, minimizing movement of room air contaminants into the chamber.

UV light is isolated by the plenum cover to protect cell cultures. Airborne contaminants are eliminated by an automatic, factory-set 5-minute UV cycle; the cycle is programmable from 0-30 minutes. Trace contaminants that attach to interior surfaces are destroyed by the passive germicidal properties of the inCu saFe™ surfaces.

EXPLAINING UV CONTAMINATION CONTROL

Some researchers can be confused with the design and penetration of UV within the chamber and therefore they may assume that the UV will affect their valuable cell cultures. The following explanation can be used to describe how our Safecell UV system does not affect their cell cultures;

ULTRAVIOLET PROTECTION

Because cell cultures are incubated in clear media vessels, the use of conventional ultraviolet light for contamination control

Contamination.	Controlled.
Downtime.	None.

UV Lamp Program Options

Mode	Function
After Door Opening	UV lamp automatically ON for five minutes after door is closed. Time factory set, user programmable from 0-30 minutes.
OFF	If UV protection is not desired.
Continuous ON	Useful for overnight decontamination prior to first use or following total chamber wipe-out after maintenance or service.

in the cell culture incubator has not been possible previously. UV light will destroy cell cultures, and ozone gas emissions from the UV lamp are toxic to cells. Ozone build-up, combined with unprotected UV exposure, can create user and environmental hazards in the laboratory. And UV light cannot reach hidden interior surfaces.

SafeCell™ ultraviolet lamp generates a narrow bandwidth emission of 253.7nm which is toxic when directly applied to microorganisms in plenum air and humidity pan water. Because this emission is outside the 185nm bandwidth that generates ozone, no ozone toxicity is present anywhere in the incubator and cell cultures remain unaffected.

Because the UV lamp is visibly isolated from the cell culture chamber by a plenum cover, UV sterilization of air and water remains in process while cell culture continues uninterrupted. The UV cycle is factory set to glow for 5 minutes following each door opening, which is sufficient to destroy contaminants during normal operation. The lamp ON time is programmable from 0 to 30 minutes depending on user preference.