Tips for Establishing Successful Cell-Based Assays: Part 2

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Standard Cell Culture Practices to Reduce Contamination Risk

Most laboratories have rules pertaining to blood-born pathogens. These rules may include mandatory training and certification prior to handling cells, human or non-human, primary or transformed. Be sure you know the rules in detail and follow them. The following is a guide for sources of contamination and ways to avoid them.

1. Culture Practices
    1. To avoid contaminating one cell line with another, don't share solutions between cell lines and don't work with multiple cell lines at the same time in the hood.
    2. Create a master cell bank very early in the culture's life as a source of cell renewal in the event of contamination down the road.
    3. Don't use antibiotics in the culture medium unless they are used for selection pressure or if culturing primary cells. The use of antibiotics can mask poor culture techniques thus a contamination can go un-detected.
2. Hood
    1. When moving items into a hood, wipe the object down with 70% Ethanol.
    2. Keep hood free of clutter.
    3. Clean spills promptly with Ethanol.
    4. Don't sneeze inside your hood.
    5. Wipe hood before and after each use with 70% Ethanol.
    6. Annual maintenance: Hoods should be completely cleaned at least 2 times a year. This entails washing the surface tray and grills and the area beneath them with a disinfectant such as Lysol followed by 70% Ethanol.
    7. Sash level limit on your hood: Keep the sash at the correct level as dictated by the hood manufacture. Too high will disrupt the air flow and compromise the hood sterility.
    8. Have the hoods recertified on a routine basis, at least once a year.
3. Incubator
    1. Wipe out once a month with disinfectant such as Lysol followed by 70% Ethanol.
    2. Refill water tray once a week, and clean the tray by wiping with 70% Ethanol prior to replacing.
    3. Have an incubator reserved for isolation of new cultures to prevent cross contamination. Do not mix new uncertified/untested cultures with those well validated cultures already in the lab.
4. Water Bath
    1. The water bath is one of the largest sources of contamination in a tissue culture lab. It is a breeding ground for microorganisms since it is moist and maintained at

37^0C. In addition, it can be a site for spills of growth medium thus enhancing the growth conditions.
2. Maintain the water bath by scheduled routine cleaning, at least once a month.
3. Add Clear Bath® to help reduce growth of microorganisms.
4. After each use of a water bath, rinse bottle containers off immediately.
5. One can avoid use of a water bath by warming culture medium to room temperature on the bench, if tested with your cells.
6. Some people use small metal beads instead of water, which will warm up the bottles but also need to be cleaned on a routine basis.

5. Personnel
    1. Train everyone on the proper use of the culture room prior to allowing them to use the facility.
    2. Wear gloves to protect the cells from you and you from the cells.
    3. Always wear gloves for handling flasks, pipettes and other equipment for cell culture. Change gloves as often as possible, particularly when moving in and out of the hood.
    4. Spray gloves with 70% Ethanol and air dry before entering the hood or touching the cell flasks EACH time.
    5. Use a cart when carrying stacks of plates or flasks.
    6. Wear clean lab coat.
    7. Roll up all your sleeves to keep them from touching flasks or bottles when you operate inside the hood.
    8. Maintain sterility of pipettes, flasks, plates, trypsin, serum and media by ONLY opening them INSIDE the hood and making sure they are tightly secured BEFORE leaving the hood.
    9. If you happen to touch a pipette anywhere unintended, discard it and get a new one.
    10. If you touch a bottle opening, wipe it with a 70% Ethanol swab. If contamination is suspected, discard the entire bottle immediately.
6. Cleanliness
    1. Bleach vacuum flasks and lines following EACH use. 10% bleach final concentration and empty the flasks WEEKLY even if not full. Keep 10% bleach of the total flask volume in the empty flask to reduce contamination.
    2. Empty bio-waste containers regularly. All waste should be double bagged.
    3. Bleach any tubes, flasks, and dishes that have contained cells. The bleached media can be washed down the sink after it has turned clear. However, <u>do not open contaminated containers</u> in the main lab area.
    4. Fluid delivery lines and drain lines should be rinsed with 70% Ethanol followed with water after EACH use. This includes Multidrop heads, Multimek lines, MRD8 lines, etc. Keep an autoclaved head in reserve if possible in case of failure of the daily one. If using the Multimek to aspirate or plate cells, rinse the lines and wash station before autoclaving the wash station. Autoclaving does not apply to heads used for 384-well delivery.