**Appendix I**

**Corrective and Preventative Action Procedures Guidance sheet : Contamination**

**What do I need to consider?**

1. What equipment have I used?
2. Which BSC(s), pipette fillers, pipettes, microscopes, water baths, and incubators have you used?
3. Do we need to quarantine the lab and who will be responsible for this?
4. Have you cultured this material before, in which lab(s)?
5. Has it spread? Who else is at risk? Do we need to extend quarantine area?
6. Who have you worked with?
7. Did it come from a vial or the cryobank (had it been previously banked?). If so, who else uses this cryobank? Who else is at risk? – what do we need to thaw and test.?
8. Cryostores – which banks/racks at risk?
9. Do we need to restrict access to the lab? Do we have enough disposable lab coats?
10. Have the Quality Manager and PI been informed?
11. Is there a common link? Repeat infections?
12. Can we confirm it has been dealt with? (monitoring?)

**How do I deal with a Contamination?**

1. Do I know what the organism is (e.g. rod-shaped motile bacteria, or fungal hyphae)?

Yes – destroy (step 2)

No - Take a Sample

* 1. Take a sample of the medium (or samples, if multiple flasks) and place into a sealable centrifuge tube.
  2. Wipe the outer surface of the centrifuge tube(s) with 1% Virkon, then with 70% IMS.
  3. Seal the centrifuge tube(s) with parafilm then keep them in the cold room in a secure secondary container.
  4. Ensure the container and tube are properly labelled with the date, user, and cell line identity and that the contents are contaminated.

1. Dispose of samples/flasks - Use an autoclave (cycle 5), or submerge samples/flasks in 1% Virkon and leave overnight.
2. If culture solutions (e.g. culture medium, wash solutions, enzyme solutions) remain unused, then sample them under quarantine (in a Biological Safety Cabinet), place 5 ml of each into sterile T25 flasks, then put them in an incubator. Inspect the flasks the following day for signs of contamination.
3. Change your lab coat (or change all lab coats if major contamination (mycoplasma) is detected).
4. Contain the laboratory area and prevent other users from using the same equipment (e.g. pipettes, pipette fillers, biological safety cabinets).
5. Inform laboratory management/PI/Lab users.
6. It is recommended that you discard any opened and unused culture solutions (including growth medium, enzyme solution, saline solutions, etc), pipette tips, tissue-culture flasks.
7. Deep clean of equipment used with 1% Virkon, followed by 70% IMS:
   1. Pipette fillers (including filter change)
   2. Pipettes
   3. Microscope stage
   4. Biological Safety Cabinet(s)
   5. Other items (e.g. shaker plates, cell counting equipment)
8. If a major contamination (e.g. mycoplasma) is suspected, a deep clean of the laboratory and a full investigation (CAPA) is necessary.
   1. Internal PCR testing (mycoplasma)
   2. External testing (mycoplasma)
      1. Unknown samples may be sent externally for identification.
9. Establish a monitoring period for future culture. Does the contaminant reappear? If so, consider that the cell bank(s) themselves may be contaminated.
10. Photograph what you see (e.g. camera-phone or microscope images), to help determine whether contaminants are repeatedly occurring.

**Sources of contamination**

1. The number 1 source of contamination is always the operator.

We harbour numerous bacteria, fungi and other microbes in and on our bodies. Without good cleaning and aseptic technique, these microorganisms will enter your cultures. Are you unwell? Is being in a rush resulting in lax aseptic technique? Do you feel sufficiently trained and confident in your aseptic technique?. Is the laboratory over capacity?..Has hair been tied back /facial hair covered?

1. Culture vessel(s)

The outer surface of flasks/plates will pick up environmental contaminants over time, which can enter the vessel if surface sanitisation is not performed properly. How thoroughly do you wipe down your culture vessels with Chemgene?

1. The laboratory environment

Dust and particulates are a ready source of microbes shed from humans and entering from the outside world. Mud and dust from roads, streets and fields adhere to clothes and boots alike. Collectively, the entire laboratory environment is contaminated to some degree. Have you sanitised your working environment properly? Have you checked that the biological safety cabinet is operating properly? . Is the air flow in the laboratories working correctly?

1. Liquid media (e.g. culture medium, enzyme solutions)

All liquid media, especially nutrient-rich culture media, are the perfect environment for growth of many microbes. Has the medium been prepared aseptically? Has it been sterile-filtered (note: will not remove viruses or mycoplasma). Have these solutions been used by other operators? Have you aliquoted your medium and tested it for contaminants before use?

1. Water bath

Water baths are good environments for microbial growth and exposed to the environment on a continual basis. When was this last cleaned? Has it been neglected over the last few weeks?

1. Disposables (e.g. pipette tips)

Frequently used disposables like pipette tips are repeatedly exposed to contamination even inside a biological safety cabinet. Have you disposed of old tips? Are you sure you are the only user of these tips? Would discarding them and using a new unopened pack be less risky for important work in future?

1. Cross contamination from other labs?

Other laboratory spaces may be used to culture microbial cells, or other mammalian cells. Ideally, activities should be contained within specific laboratory spaces. Has there been unusual movement of cell materials between laboratory spaces recently? .Has equipment been moved between labs ( pipettes?)

1. Material used within cultures ( metals/polymers/ceramic). Have they been sterilised correctly?
2. Is the external cell source of good quality? Are their contaminant screening tests up to scratch?