

# **AFCG Biosafety Guidelines for Analysis and Sorting of Unfixed Cells.**

## **Scope**

This document recommends procedures to be undertaken during the analysis and sorting of potentially biohazardous material on Flow Cytometers.

## **Definitions**

**A Flow Cytometer** is an instrument which measures the physical and chemical properties of particles as they pass through in a fluid stream.

**An Analyser** is a flow cytometer which has the ability to make such measurements but which deposits all of the particles into a waste container

**A Sorter** is a flow cytometer which performs the above functions but which has the option of sorting particles into receptacles by means of the application of a high voltage to the fluid stream.

**OGTR** - The [Office of the Gene Technology Regulator](#) has been established within the Australian Government Department of Health and Ageing to provide administrative support to the Gene Technology Regulator in the performance of her functions under the Gene Technology Act 2000. The Gene Technology Act 2000, which came into force on 21 June 2001, introduces a national scheme for the regulation of genetically modified organisms in Australia, in order to protect the health and safety of Australians and the Australian environment by identifying risks posed by or as a result of gene technology, and to manage those risks by regulating certain dealings with genetically modified organisms.

It is this office which sets down the following Physical Containment Levels which are equivalent to the US Biological Safety Levels (BSL1-4)

**PC2 (Physical Containment Level 2)**

**PC3 (Physical Containment Level 3)**

**PC4 (Physical Containment Level 4)**

## **Biohazardous material**

- All unfixed material of human origin.
- All unfixed material of primate origin.
- All human peripheral blood leukocytes, bone marrow, splenocytes, thymocytes, sperm cells,
- Cells from primary and immortalized cultures from humans, primates, and transgenic animals.
- All genetically modified organisms (GMO)
- Micro-organisms.
- Special care to be taken for brain tissue (prions) and lung lavage (tuberculosis).

## **Procedures**

### **Handling of biohazardous material**

- All biohazardous material should be prepared in a Class I or II Biohazard Hood
- If samples are to be transported to the flow cytometer they should be in a sealed primary container such as tubes with caps securely fastened inside a second sealed unbreakable container.
- Removal of liquid waste must be in two sealed unbreakable containers
- Removal of waste with no substantive amount of liquid two unbreakable containers one of which is sealed must be used
- For the transport of non-liquid, non-aerosol forming waste to autoclave in the same building, autoclave bag may be left partially open and placed in a sealed secondary container (bin with strapped down lid or equivalent)

### **Bio hazard containment**

- All unfixed samples which fall into the biohazardous category should be handled in at least a PC2 accredited facility. For accreditation procedure please see [www.ogtr.gov.au/](http://www.ogtr.gov.au/)

### **Physical Containment Level 2 (PC2)**

Incorporates any all work with primary human tissue, blood samples, recombinant DNA work classified as PC2 and work with any category 2 micro-organism.

**For examples of microbial risk groups and classifications see Australia/New Zealand Standard™ Safety in Laboratories Part 3: Microbiological aspects and containment facilities AS/NZS 2243.3:2002.**

### **Main Features**

- Surfaces must be smooth, impermeable, cleanable, resistant to damage by cleaning/disinfection agents
- Open spaces under benches accessible for cleaning
- Hands-free wash basin (or disinfectant dispenser)
- Storage area for protective clothing
- Eyewash equipment (or sterile eye-irrigation packs)
- Supply of disinfectants – labeled and dated
- Procedures for decontaminating spills
- Containment facilities for aerosols (Class I or II Biosafety cabinet)
- Personnel trained (records)
- Annual inspection

### **Physical Containment Level 3 (PC3) Main Features**

Incorporates work with any category 3 micro-organism. All of the above conditions plus special building requirements. See **Guidelines for Operator during sorting of PC3.**

**It is the strong recommendation by the AFCG that any material that requires PC4 containment (Risk Group 4 A/NZ2243.3:2002) should not be sorted by flow cytometry.**

### **Decontamination Procedures**

Waste material exposure to biohazard material. Final concentration of bleach in waste should be 0.6% (0.71M) when tank is at capacity volume.

Examples of instruments	Size of Tank	Amount of bleach. 2,000 -2,500 ppm. (0.2 – 0.25% of available chlorine bleach).	Minimum time in bleach
BD Calibur	4 litres	160ml of a 6% solution	30 mins
DAKO Cyan	20 litres	800ml of a 6% solution	30 mins
Coulter Elite	4 litres	160ml of a 6% solution	30 mins
LSR II	10 litres	400ml of a 6% solution	30 mins
BD Canto	20 litres	800ml of a 6% solution	30 mins
BD FACSAria	10 or 20 litres	400-800ml of a 6% solution	30 mins

### **Precautions (summary)**

- Where possible fixation of material is recommended with at least 1% paraformaldehyde (chemical fixation).
- Cover keyboard and mouse with plastic covers if available or use non-permeable plastic.
- Gloves and gown should be worn at all times when operating instrument.
- Appropriate signage.
- Avoid generation of aerosols when clearing instrument blockage e.g., when priming ensure that tube is firmly attached to instrument and not in the air.

## **Instrumentation – instrument capability to contain material**

Main considerations

1. Is the instrument an analyser or a sorter?
2. If an analyser is it a high throughput or a standard device?
3. Does the instrument have a closed cuvette or is it stream in air?

### **Risk assessment of analyser:**

- If it has a closed cuvette then there is generally no generation of aerosols.
- Old style instruments are not designed to contain material.
- Possible sources of contamination are handling at sample entry point, waste containment, Back drip of previous sample, blockage clearing and other accidental exposure.

### **Engineering controls**

- Enclosure
- Instrument safety features
- Aerosol management such as having a flexible hood and a vacuum hood
- Regular testing of aerosol containment
- Limiting exposure of personnel and environment
- Instrument placement (strong recommendation for at least PC2 certified facility)

### **Risk assessment of sorters**

Cuvette:-

- No generation of micro droplets. (Example of instrument is the BD FACSCalibur)

Stream in air sorters:-

- All sorters of this type generate microdroplets, i.e., satellite droplets, 3-7 $\mu$ m.
- Most droplets generated by a sorter are greater than 80 $\mu$ m and settle rapidly.
- Smaller escaped aerosols are a potential health risk to sorter operators and the environment if aerosols escape into the room, aerosol containment of a free standing or enclosed cell sorter must be verified by using appropriate testing methods.

**Before sorting any unfixed and potentially bio-hazardous specimens on a given instrument, it is imperative to validate that aerosols are contained during the regular sorting process and during instrument failure modes (see Appendix 2). If aerosols are detected outside of containment then the cell sorter must be modified such that no aerosols are detectable. Contacting the manufacturer of the cell sorter for instructions and information will be necessary to make any instrument modifications.**

**Testing must also be done whenever changes are made to the cell sorter that may affect escape of aerosols, e.g., installation of a new drive head or flow cell, replacement of the sort chamber door, or alterations in the aerosol management system.**

**For instruments that are placed into biological safety cabinets it is imperative that laboratories validate initially at installation the efficiency of aerosol containment of the cabinet before any potentially bio-hazardous sorting experiments are performed. Frequent re-testing and monitoring proper functioning of the cabinet is mandatory.**

### **Precautions**

It is recommended that sorting be performed by suitably trained operators.

Only highly experienced flow cytometry operators should perform potentially biohazardous sorts. The time required to obtain cell sorting proficiency on a given sorter varies, but training periods of +6 months are common. Some novel sorters do not require the complex alignment procedures required for older type instruments and laboratories do not feel a need for a dedicated instrument operator. **It is however extremely important that any operator who performs potentially biohazard sorting be trained carefully in the proper instrument operation and all relevant safety procedures, including aerosol containment testing on free-standing or compact sorters enclosed in biological safety cabinets. Strict adherence is mandatory as operator error could invalidate aerosol control measures and endanger personnel and the environment.** The operator should have previous laboratory experience and a minimum of two years of experience in flow cytometry. Ideally, this should include training in performing sorting on deflected-droplet cell sorters using non-infectious, fixed material of the same type that will contain the known biohazard, e.g., human peripheral blood mononuclear cell preparations.

### **Validation of containment system efficiency on instrument**

- Ensure operator is protected from any potential bio hazardous material.
- Validation should be conducted on a regular basis.
- Recommendation is that testing for aerosol containment be performed on the day if sorting biohazardous material is to be performed. (E.g., Glogerm® procedure can be performed while preparing instrument for sorting).

### **Assessment of material to be sorted**

- It is recommended that a questionnaire be given to potential clients about sample to include information on sample type, sample testing assessment of mode of transmission, virulence.

### **Immunization status of operator**

- If the operator is immunocompromised such as having chemotherapy or recovering from an infection then sorting of PC3 level organisms is not recommended.
- Whenever vaccination against a potential infectious organism that may be present in samples to be sorted becomes available, the sorter operator should consider vaccination. Vaccination and/or verification of

immunity against Hepatitis B virus are highly recommended.

## **PC2 requirements**

### Flow Cytometry Laboratory Procedures

1. Laboratory doors shall normally be closed when work is in progress.
2. Mouth pipetting, eating, drinking, application of cosmetics or smoking is prohibited. Storage of food or drink in the laboratory or any storage unit containing GMOs (e.g. refrigerator) is prohibited.
3. Laboratory gowns and gloves shall be worn during work and removed before leaving the laboratory. Closed footwear shall be worn. Hands shall be thoroughly washed when leaving the laboratory.

### **Decontamination**

4. All biological waste shall be steam sterilised before disposal.
5. Equipment used for handling cultures or contaminated material which is not readily steam sterilised shall be chemically disinfected after use (e.g. sample lines). Refer to AS/NZS 2243.3 for appropriate decontamination procedures.
6. Work benches and surfaces shall be decontaminated with a disinfectant solution after spills and when work is completed.
7. All technical procedures shall be performed in a way that minimises the creation of aerosols. In particular, operations such as sonication or vortexing which may generate aerosols are to be done in a biological safety cabinet. A period of at least five minutes shall be allowed for aerosols to settle before opening homogeniser or sonicator containers in a biological safety cabinet.
8. Each laboratory shall be equipped with basin mixers for hand washing, preferably foot-operated, elbow-operated or electronically operated. Emergency drench showers and eyewash stations shall be provided in accordance with AS/NZS 2243.1.
9. The laboratory should have easy access to biological safety cabinet if significant quantities of aerosol are to be produced. The biological safety cabinet shall comply with the following Australian Standards:
  - AS 2252.1: Biological safety cabinets (Class I) for personnel Protection and environmental protection; or
  - AS 2252.2: Laminar flow biological safety cabinets (Class II) for personnel, environment and product protection.

## **Guidelines for Operator during sorting of PC3**

**When possible, sorting of unfixed biohazardous material should be performed in a safety enclosure that provides PC3 containment. (See Appendix A). Where an enclosure is not available then the operator should comply with personnel PC3 guidelines as described in this document.**

### **Laboratory Procedures**

1. All aerosol-producing equipment such as that for sonication and vortexing shall be kept and used in the biosafety cabinet.
2. The biosafety cabinet and/or the laboratory shall be decontaminated with formaldehyde gas or H<sub>2</sub>O<sub>2</sub> after major spills of contaminated material.
3. No other work is to be done simultaneously with work requiring PC3 containment.
4. While work is in progress a sign on the door shall indicate the level of containment required for that work.
5. Laboratory clothing shall be laundered regularly. Protective clothing shall not be worn outside the laboratory; it shall be transported to the decontamination area in sealed bags or boxes. Boxes shall have provision for penetration of steam during autoclaving. Personal clothing and effects shall be kept in storage facilities located adjacent to the laboratory area and shall not be taken into the laboratory.
6. No one may enter the laboratory for cleaning, servicing of equipment, repairs or other activities unless the principal investigator or the biosafety officer has been informed and laboratory surfaces have been disinfected.
7. All equipment specified for PC3 requirements shall be dedicated to that use and area.
8. The laboratory doors shall be locked when the room is unoccupied.
9. Where a pressure steam steriliser (autoclave) is not available within the laboratory, laboratory wastes shall be bagged and placed in an unbreakable container with a secured lid for transport to the pressure steam steriliser. The surface of the container should be decontaminated with a suitable disinfectant. Wastes shall not be stored outside the facility before they are sterilised.

### **Laboratory Planning and Construction**

10. The laboratory shall not be located adjacent to or open onto, corridors used by the general public.
11. As detailed below, the laboratory is required to operate at a reduced air pressure. To maintain this pressure during access to the laboratory, an airlock shall be provided at the entry.
12. The airlock shall be fitted with two outward opening doors in series, each fitted with glass viewing panels and automatic door closers. The outer door shall be fitted with a security lock.
13. The structural design of all surfaces of the laboratory including windows shall allow for all air pressure loads imposed by the ventilation fans during normal and restricted inlet operation.
14. The construction and finish of all of the room surfaces shall be selected to ensure substantially airtight construction. These surfaces, including

those of bench tops and cupboards, shall be smooth, impervious and selected to resist attack by all decontaminating liquids, gases and agents used in the laboratory. Benches, cupboards and engineering services shall be either sealed to the room surfaces or mounted on stand-offs thus permitting wipe down access for decontamination. Windows in the laboratory shall be closed and sealed.

15. The use of false ceilings and inaccessible spaces shall be avoided.
16. Accessible voids such as roof spaces around the laboratory shall be protected against inadvertent access which could cause structural damage or penetration of the barrier.

### **Laboratory Ventilation**

17. A ventilation system that establishes a negative pressure in the laboratory shall be provided so that there is a directional airflow into the working area. Where laboratories have supply air systems, the supply air and exhaust systems shall be interlocked to ensure inward airflow at all times. The proper directional air flow into the laboratory shall be verified by airflow tests.
18. The laboratory shall be maintained at an air pressure of at least 50 Pascals below the pressure of adjacent rooms when both doors of the airlock are closed. When either door is open, this pressure shall remain at least 25 Pascals below that of the adjacent rooms.
19. The pressure differential shall be achieved by means of an independent room exhaust fan discharging to the outside atmosphere through a filter. A variable speed drive on the exhaust fan is preferred to facilitate manual or automatic room pressure control.
20. Replacement air to the room shall be drawn through a filtered aperture which is adjustable to assist in setting up the reduced room pressure. The replacement air filter shall be a Type 1 Class A or Class B complying with AS 1324 and having a minimum arrestance efficiency of 90 percent when tested in accordance with AS 1132.5 with Test Dust No. 2.
21. The exhaust filter shall be a HEPA type as specified in AS 1324. This filter shall be fitted with a prefilter having the same specification as the replacement air filter. The HEPA filter shall have metal separators. Fluid or grease seals shall not be used. Access shall be provided to facilitate determination of the integrity of the HEPA filter installation in accordance with AS 1807.6. Magnehelic gauges shall be provided to monitor separately the air pressure drop across the prefilter and HEPA exhaust filters.
22. A Magnehelic type differential pressure gauge shall be provided within the laboratory to indicate room negative pressure. Other air conditioning control switches and the exhaust fan speed setpoint control shall be located adjacent to the gauge. An audible alarm to indicate loss of room pressure control shall be provided.
23. Where the exhaust ventilation rate is inadequate to offset room heat loads, supplementary cooling in the form of a fan coil unit may be used, using chilled water or refrigerant as the cooling medium. Particular attention shall be taken in the positioning of this unit in the room in order to avoid airflows likely to disturb the operation of the biosafety cabinet.

### **Biosafety Cabinet**

24. A biosafety cabinet of the type specified for PC2 containment shall be provided. Installation of the cabinet shall comply with the requirements and recommendations of Australian Standard AS 2647: Biological safety cabinets - installation and use. This standard also makes reference to airflow disturbances in front of biological safety cabinets.

### **Decontamination**

25. Provision shall be made to decontaminate the biological safety cabinet(s) and the room independently with formaldehyde gas, and for the gas to be purged safely to atmosphere upon completion of the procedure.
26. Decontamination of the safety cabinet(s) shall be performed in accordance with the requirements of Australian Standard AS 2647 Appendix C and will require the provision of a front cover plate and exhaust duct adaptor to fit the particular cabinet.
27. Decontamination of the room will require a close-off damper in the discharge from the exhaust filter and means of closing the room replacement air aperture. Remotely switched power points facilitate the safe generation of the formaldehyde gas.
28. A pest control program should be in place.

### **Signs**

The entrance doors shall be clearly labelled with signs indicating the type of facility and the containment level. These signs are available from the OGTR only after the OGTR has approved certification of the facility.

**Authors note: This document sets out guidelines for laboratories undertaking flow cytometry. It is to be hoped that due consideration of its recommendations will be taken into account for those seeking to establish a new laboratory or upgrade an existing one especially if undertaking high-speed sorting. It should be noted that it is largely based on the ISAC guidelines document by Ingrid Schmidt et al and on the recommendations of the Office of the Gene Regulator.**

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## Appendices

### A Examples of Class I Biohazard Hood containing high speed sorter



## B. Summary of Basic PC2 practices

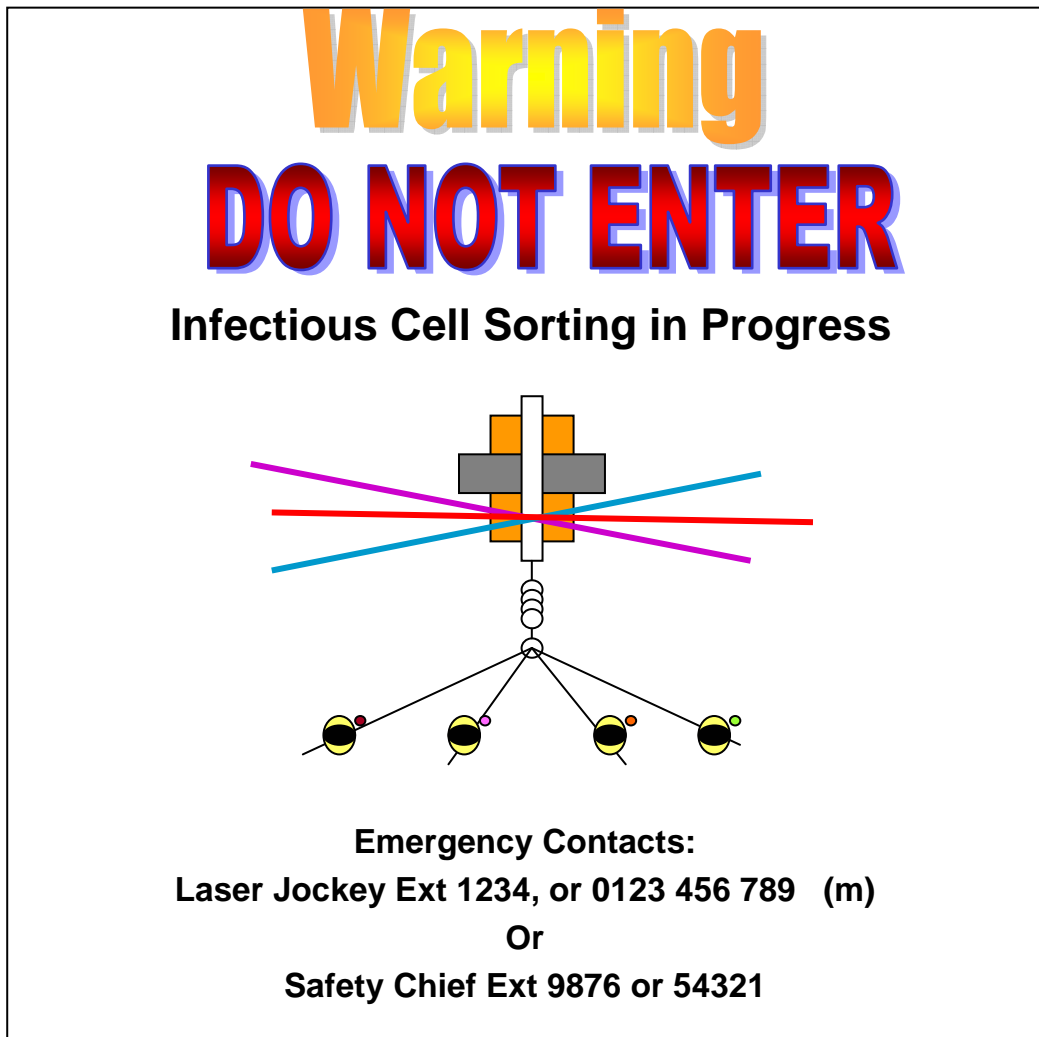
### **PC2 basic practices**

**YOU MUST COMPLY WITH ALL THE GENE TECHNOLOGY REGULATOR'S REQUIREMENTS FOR THIS FACILITY EVEN IF YOUR WORK DOES NOT INVOLVE GMOS**

- **Wear required protective clothing & enclosed shoes.**
- **Decontaminate work benches, surfaces & equipment after any spills & when laboratory work is completed.**
- **Ensure all waste is decontaminated by the appropriate method.**
- **Decontaminate all work surfaces & equipment where maintenance is to be carried out.**
- **Do not bring food or drink into the facility.**
- **Remove protective clothing & decontaminate your hands before leaving the facility.**
- **Report all unintentional releases of GMOs to the OGTR as soon as practicable.**
- **Transport GMOs in accordance with the Regulator's *Guidelines for the Transport of GMOs* (June 2001).**

**YOUR RESPONSIBILITIES ARE DETAILED IN THE CERTIFICATION INSTRUMENT FOR THIS FACILITY & OGTR GUIDELINES FOR PC2 FACILITIES**

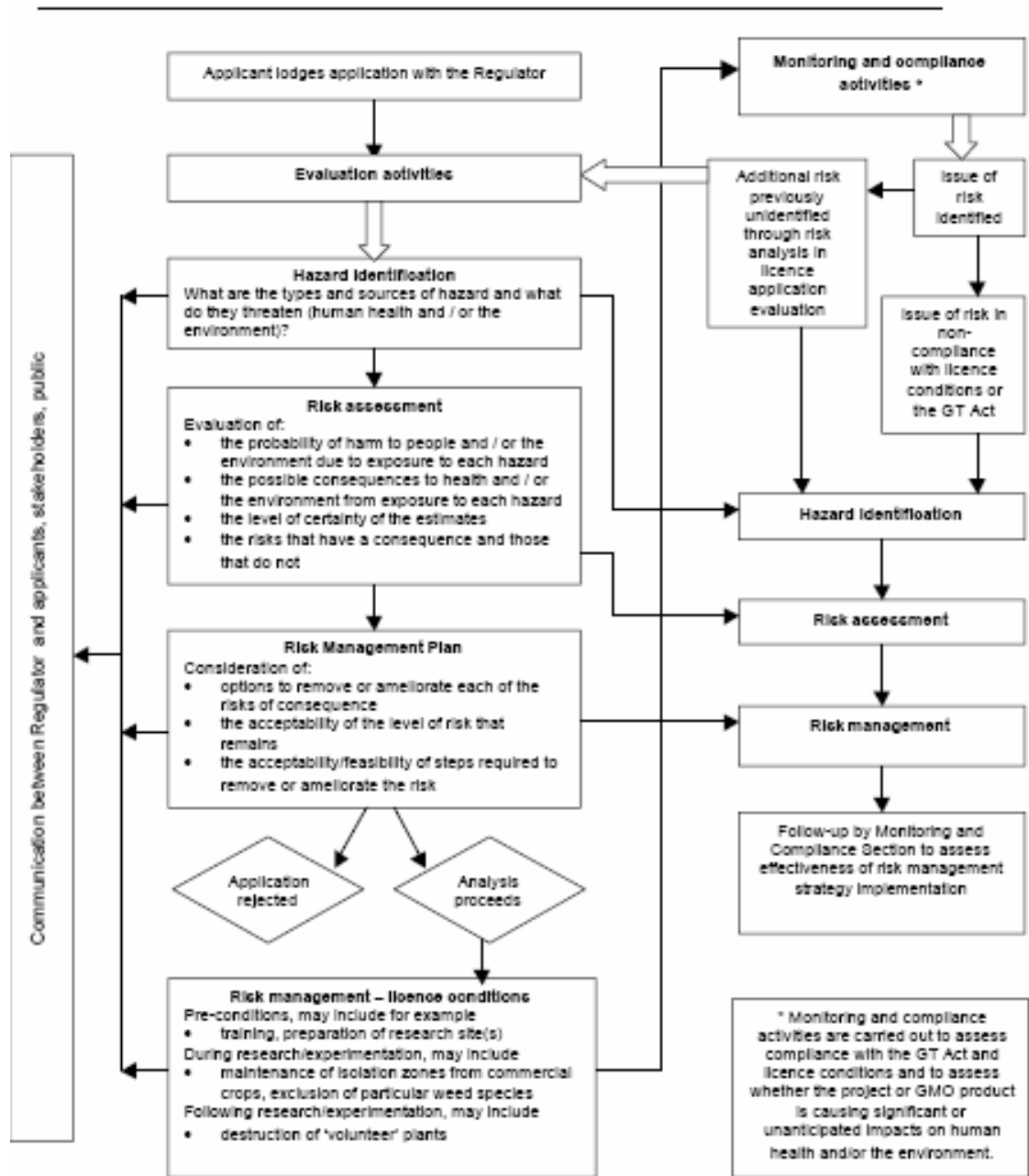
**C. Example of warning sign to be placed outside facility**



**D. PC2 Accreditation posted on door of flow cytometry facility**



## E. Risk Assessment Flow Chart



**Figure 2:** The risk analysis framework of the OGTR, indicating the linkages between the evaluation staff and the Monitoring and Compliance Section in informing the processes of each.

- indicates the next link in the process
- indicates where Evaluation processes inform those of Monitoring and Compliance

## REFERENCES

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