Guidelines for Sample preparation

All samples must be prepared as single cell suspension and filtered through a 40μm mesh to prevent clogs. (cell strainer options - cat # 352235, or cat # 352350 BD Falcon).

Only 12 X 75mm tubes (BD Falcon 352235 or 352052, 352058) may be used on the Gallios Analyser. Ideally samples should be around $1X10^7$ cells/ml for analysis.

Typically samples are prepared in PBS 1-3% FCS, or <=0.2% BSA prior to analysis. Alternatively staining buffer can be purchased from BD Biosciences (cat# 554656 – note contains Sodium Azide which is toxic and not recommended for cell sorting).

For sorting, cells may be up to $5X10^7$ cells/ml concentration for non-adherent cells (lower concentrations will result in slower sorts). Filtered samples should be supplied in 12X75mm polypropylene tubes (BD Falcon 352063) along with a supply of collection medium.

For multicolour analysis or cell sorting proper controls for compensation must be supplied. These include an unstained sample along with single color control tubes. Antibody capture beads may be used and are preferable to cells for setting compensation. Ask facility staff for more details.

Biohazardous samples

As per Mount Sinai and SLRI biosafety policies, procedures for handling biological specimens conform with practices outlined in *Laboratory Biosafety Guidelines* (3rd edition, 2004, Public Health Agency of Canada-PHAC and enforced by the human Pathogens and Toxins act.

Please note: A this time the SLRI is not able to sort live class II biohazardous samples – RG2 (e.g. all virally infected cells or cell lines of human origin). The SLRI flow cytometry Core is awaiting the arrival of an advanced cell sorter capable of handling Class II biohazardous samples (spring 2012).

Currently No provision exists to sort or analyse specimens exceeding RG2 criteria.

As part of the registration process for new clients a Biosafety questionnaire must be competed and approval granted prior to the initiation of work. This includes work to be done on analysers as well as sorters.

Please submit signed Biosafety questionnaires to *Michael Parsons* rm. 983 SLRI.

As part of the approval process a follow-up interview may be required if clients will be working with specimens meeting RG2 classification. (*For clarification of what constitutes RG2 please see Biosafety addendum*).

Specimens meeting RG2 must be fixed using a suitable method prior to analysis. (*For a list of fixation see section on fixation methods below*). Prior to commencing work the SLRI Flow Cytometry core may request a test involving the use of a *cell-viability* dye to ensure the efficacy of fixation.

Unfixed or live RG2 specimens shall be analysed with operator assistance on the Astrios cell sorter with containment. Under special circumstances provisions for analysing live RG2 specimens on analysers may be granted with prior approval.

Handling of all RG2 samples such as human blood/cell lines or infected specimens must be done in an FCF certified class II Type II BSC.

Clients are asked to remove any biological waste for disposal in their home laboratory. As a shared facility this is both a precaution for staff using the facility as well as a requirement for maintaining a sterile sorting environment.

Gloves may not be worn when using workstation keyboards.

Fixation Methods

Fixation should immobilize antigens while retaining cellular and subcellular structure. It should also allow for access of antibodies to all cells and subcellular compartments. The fixation method used will depend on the sensitivity of the epitope and antibody themselves, and may require some optimization. Fixation can be done using crosslinking reagents, such as methanol free formaldehyde (MFF). These are better at preserving cell structure, but may reduce the antigenicity of some cell components as the crosslinking will obstruct antibody binding. Another option is to use organic solvents. These remove lipids while dehydrating the cells. They also precipitate proteins on the cellular architecture.

Remember controls should be treated similarly.

In most cases a 4% final concentration of MFF will preserve biological characteristics while reducing Biohazards. It can also be used in conjunction with subsequent permeabilization methods. For best results cell surface staining is usually performed prior to fixation. Lower concentrations of formaldehyde may be used if problems are encountered.

Egg. Following surface staining add 65ul of a 10% v/v MFF stock solution (Polysciences) to a 100ul test for 15 – 20 min. Cell associated viruses may require overnight fixation. The MFF stock solution can be stored up to 6 months in the dark. Wash the cells and resuspend in stain buffer prior to analysis.

There are also commercial fixatives such as those offered by Invitrogen (Cat #GAS001S100) BD Biosciences (Cytofix/Cytoperm Cat# 51-2090KZ). These solutions also contain formaldehyde and are typically used in conjunction with permeabilization methods.

Methanol, Ethanol or ethanol/acetone mix can be used for fixation typically in conjunction with cell cycle analysis using a DNA dye such as PI. When used in conjunction with cell surface antigen staining ethanol or acetone is commonly used while methanol is commonly used for subsequent staining of intracellular antigens.

Addendum: Classification of Biohazardous samples

Risk assessment must take into account that aerosols will be generated and is based on pathogenicity and route of transmission. Of particular concern are modifications that result in expression of a toxin, know oncogene or change in tropism (host range). RG1 containment is designated for agents not known to cause disease in humans. RG2 is designated for pathogens which are known to cause disease in humans but can be contained using safety precautions. RG3 and RG4 are beyond the capability of SLRI facilities and are strictly forbidden.

An extensive list of Biological agents and their Risk group classification can be found at Public Health Agency of Canada. http://www.absa.org/riskgroups/

Appendix 1 table #1contains recommended Biosafety containment levels for selected agents. Table 2 contains laboratory practices associated with Biosafety levels.

All laboratory manipulations that can generate aerosols (vortexing etc.) should be done in a class II Biosafety cabinet. In recognition of the fact sorting generates aerosols, which can dramatically elevate biohazard risks, extra precautions must be taken with specimens classed as level II hazards. For this reason ISAC recommends sorting be done in a Biosafety enclosure.

In practice unfixed human specimens such as human blood and primary cell lines must be treated as potentially carrying blood-borne pathogens. This also applies to established human cell lines as per ATCC (American Type Tissue Collection) recommendation as well as well as cell lines that are in-vitro or animal-passaged human explanted tissues transformed by spontaneous mutation or laboratory infection with immortalization reagent e.g. Epstein Barr

virus. Only rigorously characterized human cell lines tested in a battery of tests and proved to be devoid of blood-borne pathogens may be excluded. In practice this is rarely the case, hence all human cell lines are treated as potentially having blood borne pathogens. Even samples that are fixed should be treated cautiously since there is some question about the effectiveness of fixation in samples with high titres of known virus or unknown infectious agents possibly resilient to inactivation.

Other considerations

The need to perform sorting or analysis of RG2 specimens should be reviewed on a case by case basis between the client, core manager, operator and SLRI Biosafety office. Alternatives should be explored such as the use of magnetic beads performed in a BSC. Sorting of cells labelled with radioisotopes is prohibited. General RG2 Biosafety procedures apply which includes the wearing of a lab coat (not to be removed from lab except for cleaning), gloves, and use of keyboard membrane covering- which can be wiped down, closed toed shoes. All centrifugation to be done in aerosol sealed adaptors and tubes are to be manipulated in a certified BSC (Biosafety cabinet).

All surfaces of the instrument including covered keyboards shall be wiped down with appropriate disinfectant.

Records and logs of BSL II usage shall be kept.

Appropriate warning signs shall be posted outside of the laboratory.

Biosafety Level Reference Table

Sample	Risk Group	Analysis	Viable Cell Sorting
Primary cells or cell lines derived from human or non-human primate	RG2	*Fixed-all analysers Live - operator assisted Astrios /BSC	Operator assisted Astrios/BSC
Cells derived from animals xenografted with human or non-human primate	Presumed RG2	*Fixed- all analysers Live operator assisted	Operator assisted Astrios/BSC

primary cells or cell lines		**Astrios/BSC**	
Primary cells or cell lines **from animals other than** **humans or non-human primates**	**RG1**	**Live** **All analysers**	**All sorters**
Cells from infected human or animal	**If Known as RG2**	***Fixed - all analysers** **Live operator assisted Astrios/BSC**	**Operator assisted** **Astrios/BSC**
Cells from infected human	**RG3/4 or unknown**	**Not permitted**	**Not permitted**
Genetically modified **Human or animal cells**	**Retroviral, Lentiviral or other potentially infectious vectors meeting RG2 criteria**	***Fixed - all analysers** **Live operator assisted Astrios/BSC**	**Operator assisted** **Astrios/BSC**
Yeast or Bacteria	**RG1 or RG2**	**Prior approval**	**Prior approval**