Queen Mary University of London

Occupational Health & Safety Directorate

Conducting Fluorescence Activated Cell Sorting and other Flow Cytometry studies safely

Introduction

1. Analysis of live, unfixed cells and samples using flow cytometry instruments has increased in biological and medical research in recent years. Some instruments (e.g. Fluorescence activated cell sorting, FACS) use high speed liquid propulsion technology under pressurised conditions to sort cells. This relies on droplets and aerosol (particles between 0.1 – 60 µm) creation to achieve efficient cell sorting.

2. If aerosols and droplets are not contained, instrument operators and others around can be exposed to harmful biological agents or materials. Larger particles in the range of 3 – 7 µm can be deposited into the trachea and pharynx area and ≤3 µm particles can enter the lungs. Droplets or aerosols that fall onto surfaces can be transmitted to broken skin or mucous membranes by touch and also ingested.

3. Biological material such as blood, body fluids, cells and tissue samples may contain adventitious infectious agents such as bacteria, viruses (e.g. HIV, Hepatitis B) and harmful substances such as bio toxins.

Regulatory Background

4. The Control of Substances Hazardous to Health (COSHH) 2002 Regulations[1] defines the term 'biological agent' as any agent that may cause infection, allergy or toxicity and therefore poses a hazard to human health.

5. Therefore the term 'biological agent' indicates not only the named microbial agents listed in the 'Approved List of Biological Agents[2]' but also includes cell cultures, blood, body fluids and tissues.

6. COSHH requires the assignment of a provisional Hazard Group (HG) to a biological agent (blood and body fluid samples, cells, tissue samples) if an Approved HG Classification is not available *(see COSHH definitions of HG at reference 3 below)*.

7. COSHH Schedule 3 contains Containment Level measures for handling biological agents. It requires that HG1 biological agents be handled at Containment Level 1. Likewise, HG2 or provisional HG2 biological agents must be handled at Containment Level 2 (CL2).

8. COSHH also specifies that, if aerosols are produced, infected material (at CL2 or higher) must be handled in suitable containment equipment as indicated below:

Infected material, including any animal, is to be handled in a safety cabinet or isolator or other suitable containment.	At CL2: Yes, where aerosol produced.	At CL3: Yes, where aerosol produced.

9. It is also noted in the draft Biological Agents Contained Use Regulations 2010[4] guidance that 'specific measures to control aerosol dissemination is required so as to minimise' at CL2 and 'required so as to prevent' at CL3.

10. It is the duty of a researcher under the Management of Health and Safety at Work Regulations 1999[5], to risk assess their hazardous work and under COSHH, for a

biological agent to be assigned a HG and to handle the agent under the required Containment Level and safety measures.

11. The Advisory Committee on Dangerous Pathogens (ACDP)[6] and the Scientific Advisory Committee on Genetic Modification (SACGM)[7] recommend that work with blood, body fluid, cell lines and tissue samples that are not fully characterised or authenticated are conducted at a minimum of Containment Level 2.

12. Further College Guidance on assigning a HG for biological agents and suitable containment measures are detailed in 'Procedures for working with Biohazards' (QM/H&S/0089), particularly sections B02 'Selecting an ACDP Hazard Group for biological materials' and B03 'Selecting a Containment Level for cell cultures'.

http://qm-web.safety.qmul.ac.uk/procedures/#hazard

Safety issues during operation of Flow Cytometry Equipment

13. As required by COSHH, Flow Cytometry equipment must be able to contain and minimise / prevent release of aerosols from HG2 (or higher) biological agents. Due to the method of operation, FACS equipment in particular, is prone to release of aerosols from high pressure liquid jets, which may be accentuated during blockages / failure modes. Operators and others around can be exposed to infectious or harmful aerosols that may be inhaled or transmitted to the body by touch / ingestion.

14. Microbiological Safety Cabinets (MSC) are tested and validated in the UK according to the standard EN BS 12469 / BS 5726[8,9]. ACDP guidance[10] notes that this standard must be achieved for MSC's used at CL2 or higher. Under this standard, an Operator Protection Factor of 1×10^5 or greater must be achieved (i.e. less than 1 particle in 100,000 should escape the containment).

15. It is important that flow cytometry instruments are validated to show sufficient operator protection from aerosols or droplets. The International Society for Analytical Cytology has detailed a number of methods to test aerosol containment on cell sorters in an article authored by Schmid I *et al*[11]. It should be noted that these methods may not be able to validate the instrument to the operator protection factor noted in (14) above.

Risk Assessment

16. The risk assessment of the hazardous material, assignment of HG, application of required containment level measures, provision, validation and maintenance of equipment with required safety features, suitable laboratory facilities, trained and competent operators, emergency and validated disinfection procedures must be in place to ensure the safety of operators and others.

17. The researcher intending to analyse biological samples must have conducted a suitable and sufficient risk assessment and established the HG for the material. Factors that would inform the risk assessment include pathogen virulence or attenuation, genetic modification, infectious dose, route of exposure, titre or quantity, presence of adventitious agents, type of patient / volunteer cohort that the samples were obtained from, toxic products or chemicals present.

18. A survey form or record of samples can be established by the instrument operator to prompt identification of the hazards present and provision of the risk assessment. This will also provide the operator of a record of the type of samples analysed.

19. The assignment of the HG informs the Containment Level measures required (engineering, procedural and personal protection). See 'Procedures for working with Biohazards' (QM/H&S/0089) for further details on Containment Level requirements.

Working at Containment Level 2

20. For HG2 assigned samples, the laboratory should conform to CL2 measures including restricted access to authorised personnel only, infectious aerosol prevention / minimisation by use of suitable containment, bench surfaces that are impervious to water and resistant to chemicals and disinfectants, suitable personal protective clothing and equipment such as lab coats, gloves and respiratory protection, specified validated disinfection procedures, inactivation of waste by validated methods (e.g. autoclaving, incineration, validated disinfection), hands-free operable hand wash facility at lab exit, safe storage of material, records of training to show competent workers.

21. *Only if required by the risk assessment*, the laboratory should be negatively pressurised relative to the immediate surroundings. This feature *may* be required for the handling of a biological agent that typically has an air-borne route of transmission (e.g. *Neisseria meningitidis, Bordetella pertussis*).

22. Howie-style or side / back fastening lab coats, non-latex disposable gloves with sufficient dexterity, suitable eye protection (e.g. spectacles, goggles or a visor that would prevent splashes of liquid or droplets), wearing of suitable disposable or re-usable respiratory protective equipment (minimum FFP3 type[12]) are recommended for operators and others close by during operation of instruments.

23. The instrument should be validated to provide sufficient operator protection as noted in (14 and 15) above. Suitable maintenance, decontamination regime and schedule must be in place (a standard operating procedure can detail these aspects). Emergency procedures including contact details of first aid and medical help, suitable emergency equipment and disinfectants should be detailed.

24. Suitable safety features such as an interlocking chamber door, a sorting chamber designed to contain aerosols, a system(s) to remove aerosols away from the sorting chamber safely (e.g. a vacuum line filtering device with HEPA filtration) should be provided with instruments known to create aerosols. Required safety features must always be in place during operation of the instrument.

25. At CL2, if it is not possible or reasonably practicable for the aerosol generating work to be conducted within a suitable cabinet or enclosure, **the application of other implemented measures** (see above in points 20-24) must be **justified** in the risk assessment to show that aerosol dissemination is prevented or sufficiently minimised so that workers are not exposed.

Working at Containment Level 3

26. For HG3 assigned samples, the instrument must be contained within a suitable microbiological safety cabinet or enclosure in a CL3 laboratory to prevent

exposure to aerosols. All HG3 material must be handled within the cabinet or enclosure. All CL3 measures must be adhered to. Derogations from CL3 must be approved in advance by the Health and Safety Executive.

27. HG4 samples are NOT permitted to be received, handled or disposed in any QMUL laboratory.

References

1. Control of Substances Hazardous to Health (Fifth edition): The Control of Substances Hazardous to Health Regulations 2002 (as amended), Approved Code of Practice and Guidance, HSE Books, OPSI, ISBN 0 7176 2981 3. http://www.hse.gov.uk/pubns/priced/l5.pdf

2. The Approved List of Biological Agents. HSE, Crown copyright 2004. http://www.hse.gov.uk/PUBNS/misc208.pdf

3. **Hazard Group 1** - A biological agent that is unlikely to cause human disease; **Hazard Group 2** - A biological agent that can cause human disease and may be a hazard to employees; it is unlikely to spread to the community and there is usually an effective prophylaxis or effective treatment is usually available; **Hazard Group 3** - A biological agent that can cause severe human disease and presents a serious hazard to employees; it may present a risk of spreading to the community but there is usually effective prophylaxis or treatment available.
4. The Biological Agents and Genetically Modified Organisms (Contained Use) Regulations 2010: Guidance ***(Draft)*** http://www.hse.gov.uk/aboutus/meetings/committees/acdp/index.htm

5. Management of Health and Safety at Work Regulations 1999. Approved Code of Practice and Guidance, HSE Books, OPSI, ISBN 978 0 7176 2488 1. http://www.hse.gov.uk/pubns/priced/l21.pdf

6. Biological Agents - Managing the risks in laboratories and healthcare premises. ACDP, HSE Books, 2005. http://www.hse.gov.uk/biosafety/biologagents.pdf

7. The SACGM Compendium of guidance, HSE Books, 2004. http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/

8. BS 5726 Parts 2 and 4 Microbiological safety cabinets – Information to be supplied by the purchaser to the vendor and to the installer, and siting and use of cabinets – Recommendations and guidance 2005. ISBN 0 580 45590 4.

9. BS EN 12469:2000. Biotechnology. Performance criteria for microbiological safety cabinets, ISBN 0 580 34869 5.

10. The Management, Design and Operation of Microbiological Containment Laboratories, ACDP, HSE Books 2001. ISBN 0 7176 2034 4

11. Schmid I, Lambert C, Ambrozak D, Marti GE, Moss DM, Perfetto P. International Society for analytical cytology: Biosafety standards for sorting of unfixed cells. *Cytometry* 2007; 71 A: 414-437

12. FFP3 masks: assigned protection factor 20 X Occupational Exposure Limit for chemicals; there are no Occupational Exposure Limits established for biological

agents. FFP (filtering face piece) Level 3 masks are the highest grading available in the UK and EU (under EN 149) for disposable respiration protective equipment. FFP3 masks should be fitted to ensure that they fit as well as possible onto the face especially around the nose and mouth particularly, taking account of the manufacturer's instructions and validated face-fitting requirements.

Document Control

Initial Data

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Status:	Version 1 – Live
Date of Issue:	August 2011

Version No.	Date of alteration and re-issue	Details of changes	Changes made by
1	March 2012	Filename/path changed from QM-HS-0099	Darren Ford