

Cell Sorting with Pathogens - Safety Precautions in Flow Sorting at Containment Level 3

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Flow cytometry staff at the HPA Porton Down site have several years of experience in providing flow cytometry services at containment level 3 (CL3). This, combined with the experience on site of biosafety and of specialist microbiological facilities, has allowed the development of a contained sorting capability. The requirement is for high speed sorting of a range of bacterial pathogens, including *Mycobacterium tuberculosis* and *Bacillus anthracis*.

After assessment of a number of flow cytometers, the MoFlo was considered the most suitable and technically adaptable sorter for housing in containment. A flexible film isolator was determined to be the only feasible option to allow CL3 work to be conducted under UK Health and Safety requirements that demand primary containment of hazards. Siting of MoFlo components, the isolator design and installation of facility services have been implemented to maintain ease of working whilst minimise the risks to the operator.

Rigorous procedures are required for the safe operation, cleaning, and decontamination of both the equipment and the facility. These steps are required to protect both the operators and the service personnel. Two non-pathogenic organisms were selected to validate the containment systems. *Bacillus subtilis* var niger was used to simulate a bacterial agent and *Escherichia coli* (NCIMB 9481) MS2 coliphage was used to simulate a virus. The expected bioburden to be released during operation and following blockage of the nozzle was determined by aerobiological assessment.

Biosafety considerations are currently being highlighted across the field of flow cytometry. Our experience in working in a specialist containment level 3 facility provides insights into issues that are relevant to cell sorter operators working with all types of samples. These approaches to containment apply generally and would address safety issues such as those involved with sorting virally infected mammalian cells, tumour cells etc.