



Risk Assessment for Genetically Modified Micro-organisms [GMMs]  
Class 1 & Class 2

*The following constitutes an **example** of a Risk Assessment for a GMM falling within Class 1. It is designed as a guide to how a Risk Assessment should be conducted, the elements which should be considered during a Risk Assessment (the recipient organism, the insert, the vector, the host, the final GMM, human health and environmental considerations, containment measures) identification of possible harmful effects and the possibility of those effects occurring. The Risk Assessment process culminates in the determination of the final classification of the GMM and the appropriateness of the corresponding containment measures.*

Description of each GMO:  Cloning of structural/non-structural gene sequences from Pseudomonas aeruginosa into E.coli XL-1-Blue using standard molecular biology techniques.
Purpose of the contained use:  To investigate the opportunistic pathogenicity capability of Pseudomonas spp.

PROCEDURE 1

(3.1) Identification of harmful properties (hazard) of the GMM:	
(3.2.1) the recipient organism	The recipient organism is E.coli XL-1-Blue, which is a commercially available E.coli strain derived from E.coli K12 and used for routine and widespread molecular biology

	<p>studies. Derivatives of K12 are avirulent and may be considered to be equivalent to Directive 90/679/EEC (and amendments) and ACDP biological agents hazard group 1 because they have an established record of safety in the laboratory with no adverse effects on human, animal or plant health or the environment. Furthermore E.coli XL-1-Blue is incapable of survival outside the laboratory.</p>
(3.2.2) the insert	<p>Inserts constitute structural and non-structural gene sequences encoding transmembrane receptor molecules, outer membrane proteins, binding proteins. These proteins will be expressed at high levels in E.coli in order to obtain proteins for functional analyses. None of these proteins are known to be involved in pathogenicity but all are anticipated to play physiological roles.</p>
(3.2.3) the vector	<p>The vectors used for this project are commercially supplied, pUC derived (history of safe use), are unlikely to endow any pathogenic traits and are rendered non-mobilisable by virtue of the fact that they lack bom, mob and tra genes.</p>
(3.2.4) the host	<p>The donor organism is <i>Pseudomonas aeruginosa</i>. It is categorised as Class 2 according to Directive 90/679/EEC and amendments. However <i>Ps. aeruginosa</i> is not considered a primary pathogen but rather an opportunistic agent involved in hospital acquired infection. It exists as a normal inhabitant of human intestinal flora and is associated with infection in the immunocompromised host. It is widely dispersed in the hospital environment, has no known involvement in environmental processes and is non-spore forming.</p>
(3.2.5) the resulting GMM	<p>The host organism is classified as belonging to Class 1. The insert and the expressed gene product are not considered</p>

	harmful or capable of altering the pathogenicity/ survivability /fitness of the recipient organism. On this basis the GMM is deemed incapable of causing disease in humans, animals or plants and does not cause adverse effects in the environment.
(3.2.5.1) Human Health Considerations	E.coli XL-1-Blue (the recipient organism) is not known to cause disease in animals or plants or cause any adverse effects in the environment. The genetic modification described will not increase the ability of the GMM to cause disease. No enhanced pathogenicity or virulence is expected.
(3.2.5.2) Environmental Considerations	<p>The recombinant E.coli will be cultured at volumes not exceeding 50ml at a time. Precautions will be taken to avoid aerosols and accidental spillages will be dealt with by swabbing with Virkon disinfectant which is bactericidal. Contaminated solid waste such as plastic disposables, tissue paper, culture plates, etc., will be collected in autoclave bags and autoclaved at 124°C for 35 mins. The autoclave is located in the building and is validated on an annual basis.</p> <p>Liquid waste such as liquid culture medium is treated by adding disinfectant to achieve a final concentration of 10% and left to stand overnight before discharge.</p> <p>Sharps material is disposed of in cin bins, which ultimately are removed by (name of waste contractors) for disposal by (method of disposal, which should be checked with the waste contractor).</p> <p>Personnel are fully trained in aseptic techniques, are required to wear white coats while working in the laboratory and gloves while carrying out aseptic techniques.</p> <p>The presence of the Ps aeruginosa characters described, in a laboratory strain of E.coli are not considered likely to</p>



	enhance its survival in the wild.
<p>(3.3) Initial classification of the GMM:  E.coli X-L1-Blue has an established record of safety in the laboratory and therefore is unlikely to cause disease to humans, animals or plants. Furthermore the genetic modification is not expected to alter this. The pUC derived vectors have a similar history of safe use and the inserts will not endow the recipient organism with a phenotype likely to cause disease or to have deleterious effects in the environment. Therefore the GMM is unlikely to cause disease or to have deleterious effects in the environment and is allocated to Class 1GMM - activities of no or negligible risk for which level I containment is appropriate to protect human health as well as the environment</p>	
<p>(3.4) Assessment of possibility of harmful effects occurring:</p>	
(3.4.1) Nature of activities to be undertaken	The experiments entail standard molecular biology techniques which carry no special risk and all are conducted in accordance with safety SOPs held in house.
(3.4.2) Concentration and scale	E.coli XL-1-Blue is cultured on LB solid media (approximately 10 petri dishes at a time) or in LB broth at typical volumes of 50ml or less.
(3.4.3) Culture conditions	Culture conditions are as per 3.4.2 above. The GMM is incubated at 37°C for 24 hours.
(3.4.3.1) Environment likely to be exposed	Only the immediate laboratory environment is likely to be exposed and as outlined in section 3.2.5.2 considerable care is taken to ensure that the GMM is contained and that areas beyond the laboratory are not exposed to the GMM. Furthermore it is expected that the GMM will not survive for significant periods in the environment.
(3.4.3.2) Presence of susceptible	Neither humans, animals nor plants are susceptible to E.coli



species	XL-1-Blue and the insert and the expressed gene product are not considered harmful or capable of altering the pathogenicity/survivability/fitness of the recipient organism.
(3.4.3.3) Whether the environment can support the survival of the GMM	E.coli XL-1-Blue is a disabled strain and it is expected that the organism would not survive in the environment.
(3.4.3.4) Effects on the physical environment	Since the GMM is considered incapable of survival in the environment no effects on the physical environment are expected.

## PROCEDURE 2

### (4.1) Determination of final classification and containment measures

E.coli XL-1-Blue is non-pathogenic, has a history of safe laboratory use and is classified as Class 1. The inserts, vectors and expressed gene product are not considered harmful or capable of altering the pathogenicity/survivability/fitness of the recipient/host organism. On this basis the GMM does not pose a risk to human/animal/plant health or the environment. Therefore the containment level required to control that risk is Level 1, the same as unmodified E.coli XL-1-Blue. The GMM activity is therefore classified as Class 1.

### (4.2) Confirmation of adequacy of final containment measures

Principles of Good Microbiological Practice and Good Occupational Safety and Hygiene in accordance with Part A of the Fourth Schedule of S.I. No. 73 of 2001. The requirements of Containment Level 1 as given in table 1A - 'containment measures for contained use of GMOs in a laboratory' - of S.I. No. 73 of 2001.