# GM Microorganism Risk Assessment

## *Full Assessment, New Format*

**PI Responsible:** Paul Crocker/Colin Watts
**Division:** Cell Biology and Immunology
**Assessor:** Hannah Richards
**Extn:** 85857
**Approver:** Ian Scragg
**Approval Date:** 18/12/2008
**Review Date:** 18/12/2009

**Serial Number:** USOGMM1123 **Version:** 4
**SS Code No:**
**Created:** 2/10/2008, 12:53:51 PM, lgrayson
**Modified:** 18/12/2008, 1:38:32 PM, lgrayson
**Assessment Complete:** ◉ Yes
**Assessment Reviewed by BSA:** ◉ Yes
**Permission Granted by HoD:** ◉ Yes

**Final Classification of Project:** **Class 2**

**Title:** **Investigating the role of siglecs in the interaction between pathogen and immune response using Salmonella Typhimurium**

**Lab:** Floor 2
**Bldg:** Wellcome Trust Biocentre

### 1: Brief Description of Project

We are interested in the biology of a family of molecules called siglecs, expressed on cells of the immune system, which bind sialated ligands. Mammalian cells carry many sialated ligands which may signal through siglecs and inhibit any immune response towards itself. Most pathogens are not sialated, however, some human pathogens have gained the ability to mimic host gangliosides, e.g. some strains of Campylobacter jejuni, which may provide a mechanism by which the pathogen inhibits the immune system. In this project we aim to explore the role of siglecs in the interaction between pathogen and immune response. Salmonella enterica serovar Typhimurium is a natural murine pathogen that does not bear sialated ligands, however a collaborator is generating genetically modified strains that will express these ligands. Both the wild-type and the recombinant strains will be used to evaluate in vitro and in vivo immune responses to defined ligands, utilising mice with altered siglec expression/function. This work involves both the propagation of Salmonella in vitro as well as the infection of mice and harvesting of tissues for analysis.

### 2: Hazards to Human Health

(a) Associated with recipient micro-organism

Salmonella enterica serovar Typhimurium is classed as an ACDP category 2 organism. It is not the serovar that causes typhoid fever. This organism can give rise to a form of gastroenteritis called 'salmonellosis' if contaminated food is ingested. Symptoms are mild in healthy adults and most severe in infants and elderly people. Infection requires antibiotic treatment commonly with ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, or ciprofloxacin, i.e. therapeutic interventions are available.

Salmonella is routinely cultured in clinical bacteriology labs on the open bench. The main hazard is that the operator's hands are contaminated and that the bacteria are transferred to other surfaces and food. The use of gloves, disinfectant, hand washing and due care and attention is sufficient to reduce this hazard to a minimum. A second hazard is self inoculation by splashes or sharps. The bacteria are only pathogenic if ingested or injected, i.e. skin contact is not an infection risk provided the area is cleaned. Sharps are used only when essential.

(b) Arising directly from the inserted genetic material (toxin, oncogene)

The GM organisms derived from wild type organisms have been genetically modified to lose certain genes involved in sialic acid metabolism and transfer onto the lipo-oligosaccharide molecule. The loss of these genes is not inherently harmful. This would however result in the generation of sialated ligands on the surface of the bacteria.

(c) Arising indirectly from the inserted genetic material (eg alteration of pathogenicity, host range, tissue tropism, mode of transmission or host range)

As a result of the gene deletion, the genetically modified strains express sialated ligands which are potentially recognised by Siglecs. Theoretically, this may result in some 'molecular mimicry' between pathogen and host and therefore the GM strains may be able to inhibit the immune response targeted against it. However, we would not anticipate that it would be to such a degree as to warrant classification above that of the wild-type salmonella. Indeed it may be that a slightly less vigorous immune response may result in reduced immunopathology without affecting bacterial control. It is also possible that recognition by other Siglecs could enhance the immune response to the pathogen resulting in more rapid clearance, since the function of Siglecs is still undetermined.

The genetic modifications made to the bacteria are extremely unlikely to affect their transmissability or susceptibility to disinfectants or heat. It is also unlikely that the modifications would alter sensitivity to antibiotics, i.e. therapeutic interventions are available.

(d) Arising from transfer of genetic material to a related micro-organism

There is no potential transfer of genetic material as the genes of interest have been deleted.

**3: Assign a provisional Containment Level**

○ Class 1/Level 1 Containment ◉ Class 2/Level 2 Containment ○ Class 3/Level 3 Containment

**4: Hazards to the Environment**

(a) Associated with recipient micro-organism

Salmonella is widely prevelant in the environment and the original strains used here have been isolated from various sources out-with the laboratory. In the unlikely event of release from containment level 2 facility, these strains pose no greater risk to the environment than the wild-type strain.

(b) Arising from genetic material

In the very unlikely event of a release from the Containment Level 2 Facility, as the GM organism is a Knock-out rather than Knock-in strain, the chance of gene transfer does not apply.

**5: Nature of Work**

(a) Brief description of nature of work (include maximum culture volumes)

Microbiological procedures will be carried out under Containment Level 2 in the general lab and tissue culture suite. For propagation purposes cultures will be grown in volumes of 25-500 mls. Cultures will also be collected for murine infection, or used fixed or unfixed in in vitro experiments. Murine infection will be carried out in a Containment Level 2 facility in the animal unit. Mice will be infected by injection of a small volume of bacteria, and infection monitored in homogenised tissues (typically liver and spleen).

(b) Is a microbiological safety cabinet or isolator required to protect against aerosol transmission?

○ Yes ◉ No

(c) Waste Disposal

Salmonella is sensitive to moist heat (121° C for at least 15 min).
Virkon is effective against S typhimurium at 1% for 10 min (efficacy data available on manufacturer's web site) and will be used for surface disinfection.

Liquid waste is collected in a sealable, robust, autoclavable container, autoclaved then disposed of to drains. If using a microbiological safety cabinet store the container within the cabinet during use and seal before removal. Do not use aspirator set-ups.

Solid waste (including plastic pipettes and agar plates) is collected in a lined, biohazard labelled, autoclavable bin, autoclaved then disposed of as normal refuse.

Large glass pipettes are not used.

Sharps waste is collected in an autoclavable sharp-safe container, autoclaved then disposed of as clinical waste. If using a microbiological safety cabinet store the container within the cabinet during use and engage temporary closure before removal. The use of sharps must be avoided unless essential.

(d) Are sharps required? YES or NO - if yes justify use.

YES - for injection of mice. No alternative.
For other activities - No.

(e) If the work involves experimental infection of animals is it known if the animal will shed the genetically modified micro-organism? If YES give details and measures to prevent exposure.

YES – can be shed to the bedding of animals and can spread to animals within the same cage by fecal matter. The risk of infection to humans is minimal as and is further reduced by: wearing appropriate PPE; housing animals in sealed, individually ventilated cages; adhering to strict disinfection and cleaning regimes; use of a Class II microbiological safety cabinet. Cage waste is removed within a Class II cage-change cabinet, double bagged then autoclaved before disposal. Used cages are treated with Sanosil Super 25 (efficacy data available on manufacturer's web site) prior to regular cage washing procedure. Carcasses are double bagged and autoclaved prior to disposal.

(f) If the work involves the experimental infection of plants what is known about the likely route of transmission of the genetically modified micro-organism?

N/A

(g) Where will the genetically modified micro-organisms be stored?

WTB Floor 2 TC suite, -80 freezer in central equipment room and liquid nitrogen cell freezer in central equipment room. Samples in liquid nitrogen cryo-stores will be contained in proper cryo-tubes & stored in the liquid nitrogen vapour phase to eliminate risk of tube explosion upon initial warming. Samples in fridges/freezers will be doubly contained. Fridges, freezers & cryogenic storage vessels will be secure, biohazard labelled & subject to a well maintained inventory system.

(h) How will the genetically modified micro-organisms be transported within/between buildings to minimise risk of spillage/escape?

Samples will be doubly contained during transport & clearly labelled with a contact name & number, the nature of the sample & the biohazard symbol. Inner container/tube will be robust & leak-proof. Outer container will be robust, leakproof & contain enough absorbent material to absorb the total volume of sample should the inner container leak.

(i) Will staff and students receive any vaccinations or health surveillance?

None required. However, all those who may be exposed must be familiar with the signs of Salmonella infection.

(j) Emergency Plan

Small scale cultures and low risk activity.

(k) Monitoring

Autoclave Testing and Maintenance
During the first four years after installation an annual 12-point validation test, employing independent thermocouples, is used to demonstrate that the autoclave holds the specified temperature and pressure for the required period of time. Thereafter, autoclaves are serviced every 6 months by a reputable service provider and calibrated annually to ensure the validation criteria are met. During normal, daily operation indicator tape and, in the case of liquid waste, a temperature probe placed at the centre of the load, are used to ensure the required conditions are achieved. Servicing and testing is arranged and test reports are kept by the CLS Health & Safety Coordinator..

Maintaining PPE
Users are required to routinely check their PPE (e.g. lab coat, safety glasses) and keep it in good order. Defective PPE must be repaired or replaced immediately. Laboratory Managers are required to ensure the appropriate PPE is readily available and keep an inspection record for non-standard PPE, e.g. that used in Liquid Nitrogen facilities.

Inspections, Audits and Continual Monitoring
Safety Inspections are carried out regularly to ensure health & safety policy & procedures are being followed and that the required risk assessments and training records are complete and up to date. Inspections are time-tabled and inspection teams selected by the CLS Health & Safety Working Group. Inspection team members are selected from CLS Health & Safety personnel and senior management. Inspection reports are submitted to the CLS Health and Safety Management Committee for review. Audits performed by an external, independent body are are arranged by the CLS Health & Safety Working Group when deemed necessary by the CLS Health & Safety Management Committee. Lab Managers and BSOs are required to continually monitor safety standards and compliance with Health & Safety Policy & Procedures, within their designated area, and report problems and non-compliance to the CLS Health and Safety Working Group.

MSC Testing and Maintenance (TC suites only)

MSCs are serviced and operator protection (KI) tested on an annual basis by a reputable service provider. A certificate of conformity to the required standard is displayed on each cabinet. The Health and Safety Information Officer is responsible for arranging the servicing schedule, ensuring fumigation/decontamination is carried out prior to testing and issuing and keeping a copy of the certificates of conformity. Users are required to perform a visual check on all alarms and indicators before each use and report any defects immediately to their Lab Manager. Note: If an MSC is moved to a new location, or equipment in a room containing a cabinet is significantly rearranged, to the extent where it may affect the airflows within the room, the cabinet must be KI tested before use to ensure operator protection has not been compromised.

Negative Pressure Testing (TC suites only)

Pressure differentials in TC suites are checked regularly to ensure the suite is at an air pressure negative to the immediate surroundings. Checks are arranged by the CLS Health & Safety Information Officer.

## 6: Final classification of project

○ Class 1 ◉ Class 2 ○ Class 3

## 7: Additional information and comments